Fouling by non-indigenous marine species – impacts on biodiversity and mariculture

by
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Declaration

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Abstract

Alien fouling species are most likely to be introduced into Saldanha Bay via two vectors: the high shipping volume experienced in the Bay and the intensive mariculture operations that take place in the system. The invasive ascidian *Ciona intestinalis* was first recorded in South African waters in 1955 and has since become a common fouling species in Saldanha Bay. Despite this ascidian being known to impact species richness elsewhere, its ecological impacts have not been considered in South Africa. The first chapter of this thesis aims to assess the impact of this species on indigenous fouling communities and considered the role of water movement and depth in moderating any effects. The results from this study revealed that water movement and depth affected settlement of *C. intestinalis,* with individuals recorded only under conditions of low water movement and only on deep experimental plates (i.e. 3.1 m depth). Unexpectedly, no effect on community structure or diversity was found where *C. intestinalis* settled. The second chapter aims to document seasonal trends in the fouling communities that affect oyster farms in Saldanha Bay, and assess the prevalence of alien species in these communities. Community structure differed significantly between seasons and depths. The orientation (i.e. the top versus bottom side of oyster cages) only affected the settlement of mussels. Deep cages supported greater fouling biomass than shallow cages. Although there were fewer alien fouling species than indigenous species, alien species supported a greater biomass. At these high densities, alien filter-feeding species may have negative impacts on cultured oysters. The last chapter follows on from this and investigates the impact of *C. intestinalis* fouling on the growth of cultured oysters, assessing the benefits of four week versus nine week intervals between cage cleaning. During this work the settlement rate of *C.*

intestinalis was unexpectedly low. Results showed that at these low abundances, this species had no effect on growth, shell density or condition of the oysters. In fact cleaning at a four weekly interval was detrimental to the growth of the cultured oysters. It is thus suggested that oyster farms maintain their current nine week cleaning regimes.

Opsomming

Die invoer van uitheemse bevuilingspesies na Saldanhabaai geskied waarskyklik via twee vektore: die groot omvangs inskeping wat in die Baai ervaar word, sowel as marikultuur aktiwiteite wat binne die sisteem bedryf word. Die indringer ascidian *Ciona intestinalis* is in 1955 vir die eerstekeer in Suid Afrikaanse waters waargeneem waarna dit 'n algemene bevuilingspesie in Saldanhabaai geword het. Al is dié ascidian elders daarvoor bekend om spesiesrykheid te beïnvloed, is die ekologiese impakte wat dit in Suid-Afrika mag hê nog nie oorweeg nie. Die eerste hoofstuk van die tesis het beoog om die impak wat dié spesie op inheemse bevuilingsgemeenskappe mag hê te beraam en neem ook verder die invloed van waterbeweging en diepte op hierdie impak in ag. Die studie se resultate onthul dat waterbeweging en diepte beide die vestiging van *C. intestinalis* beïnvloed. Individue is slegs tydens lae water beweging en op experimentele plate geleë in diepwater (i.e. 3.1m diepte), waargeneem. Daar is geen effek op gemeenskapstruktuur of -diversiteit gevind waar *C. intestinalis* gevestig is nie. Die tweede hoofstuk het beoog om seisonale patrone binne die bevuilingsgemeenskappe wat oesterplase in Saldanhabaai beïnvloed, aan te teken en om die algemeenheid van uitheemse spesies binne dié gemeenskappe te assesseer. Daar was 'n beduidende verskil in gemeenskapstruktuur tussen seisoen en diepte. Die ligging van die oesterhokke (i.e. die boonste teenoor die onderste kant van die hokke) het slegs die vestiging van mossels beïnvloed. 'n Hoër bevuilingsbiomassa was op die diepgeleë hokke teenwoordig. Alhoewel daar minder uitheemse bevuilingspesies as inheemse -spesies teenwoordig was, het uitheemse spesies bygedra tot 'n groter biomassa. Uitheemse filtreervoedende spesies kan tydens hoë digtheid potensiële negatiewe impakte vir gekweekte oesters inhou. Die laaste hoofstuk het die impak van *C.*

intestinalis bevuiling op die groei van gekweekte oesters geondersoek en het terselfdertyd die potensiële voordele van vierweeklikse teenoor negeweeklikse intervalle tussen hok skoonmaak, geassesseer. Die vestigingskoers van *C. intestinalis* was onverwags laag gedurende dié studie. Resultate het daarop gedui dat dié spesie tydens ‘n verminderde teenwoordigheid, geen effek op die groei, skulp digtheid of toestand van die oesters gehad het nie. Daar is verder gevind dat hok skoonmaak op 'n vierweeklikse interval wel nadelige vir die groei van oesterkulture is. Dit word dus voorgestel dat oesterplase hul huidige skoonmaak roetine behou.

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Chapter 1

General introduction

Marine alien species can have devastating effects on the ecology and economy of an area (Grosholz *et al*., 2000; Robinson *et al*., 2005a; Vila *et al*., 2010). Their potential to become invasive makes them a serious concern from a marine conservation perspective (Bax *et al*., 2001; Gaither *et al*., 2013). Besides those species that simply establish naturalised populations, there are marine alien species which spread from their point of introduction, to compete with and dominate the native fauna and flora, thus becoming invasive (Bax *et al*., 2003; Schwindt, 2007). The introduction of marine invasive species into foreign areas can have negative impacts on biodiversity (McDonald, 2004; Blum *et al*., 2007), as well as community structure of coastal habitats, such as rocky shores, soft bottoms in the sub-littoral zone, beaches, marshes and estuaries (Carlton, 1999). Despite the variety of habitats in which they occur, globally marine invasions are more predominant in estuaries and bays, as this is where harbours are usually situated (Grosholz, 2002; Ruiz *et al*., 2011).

Invasive alien species can act as environmental engineers, as they often alter their receiving environment (Bax *et al*., 2003). The fast growth and high abundance of such species make them important components of transformed environments (Castilla *et al*., 2004). These transformed environments can provide new habitats for other alien species, thus impacting the original biodiversity and abundance of indigenous species (Castilla *et al*., 2004; Robinson *et al*., 2007a, b). Alien species are often able to direct more energy into growth and reproduction than indigenous

species, because their natural predators, competitors, diseases and parasites are frequently absent from their new environment (Hairston *et al.*, 1960; Howarth, 1991; Kvach & Stepien, 2008). On a global scale most detailed studies on invasions of marine alien species are concerned with those species that have colonized the intertidal zone (Blecher *et* al., 2008) and this pattern is mirrored in South Africa.

Vectors of marine invasions

There are two mechanisms by which marine organisms spread. Firstly range expansion, which involves dispersion by natural processes and secondly introductions, which involve dispersion through human activities (Carlton, 1989). The prevalence of invasions of the near-shore environment has stimulated considerable research into both the vectors of marine invasions and their impacts (Ruiz *et al.,* 2000, Lewis *et al.,* 2003). Marine biofouling is defined as the unwanted accumulation of animals, plants and micro-organisms on exposed artificial surfaces immersed into sea water (Meseguer *et al*., 2004). Alien fouling species are primarily introduced via vessels and shipping related equipment (Carlton, 1989; Bax *et al*., 2003; Mineur *et al*., 2007; Mead *et al*., 2011a) and mariculture (Galil, 2007). In the past, wooden hulled ships and their dry ballast were the primary transport for alien marine species (Coutts *et al*., 2003). Although wooden hulled vessels are no longer in commercial use, wood-boring species still play a role in the fouling of wooden piles, barges and yachts (Millard, 1951; Griffiths *et al*., 2009).

Monitoring of fouling species on the hulls of ships is often not undertaken, or is at best poorly managed (Godwin, 2003). In the past, methods aimed at mitigating fouling by marine organisms were focused on the use of tributyltin (TBT) antifouling paints on the hulls of sea-going vessels (Evans *et al*., 1995; Choi *et al*., 2013). Though TBT is efficient in reducing hull fouling, its toxicity is thought to pose a risk to aquatic environments (Goldberg, 1986; Petersen & Gustavson, 2000; Choi *et al*., 2013). For this reason, global regulation has focused on banning TBT paints and has been formalised in the International Convention on the Control of Harmful Anti-fouling Systems on Ships, which came into force in 2008. Although the banning of TBT was sound from a pollution prevention perspective, the implications for the spread of alien fouling species are considerable (Minchin & Gollash 2003; Faasse & Ligthart, 2007). Since the banning of TBT, alternative substances have been used, but these are less effective at preventing fouling (Nehring, 2001). From an invasion perspective this is of big concern, as increased fouling could lead to the increase in frequency of introductions of alien fouling species across the globe.

Both ballast water (i.e. water which is pumped into a ship to assist with stability and trim) and hull fouling are now recognised as the primary transport mechanisms for alien marine species (Coutts *et al*., 2003). The fouling of ship hulls is regarded as an important international risk for marine alien species introductions, that is being poorly managed (Godwin, 2003). It has been estimated that there are up to 10 000 species of marine organisms in transit in the ballast tanks of the global shipping fleet at any time (Bax *et al*., 2003).

The increase in volume and speed of transoceanic travel during the last century has seen a rise in the rate of introductions across bio-geographic regions (Ruiz *et al.,* 2000; Mack *et al.,* 2000; Bax *et al*., 2003). The technological advancement of shipping vessels has led to their increase in size, with a concurrent increase in ability to transport marine species (Bax *et al*., 2003; Minchin & Gollasch, 2003). The increase in speed of modern vessels and the use of antifouling paint has aided the decrease of hull fouling, but this still remains an important vector for the movement of alien species (Bax *et al*., 2003; Minchin & Gollasch, 2003).

The organisms which foul the hulls of ships undergo extreme oceanic conditions and this can lead to a decrease in their metabolic functions, such as growth (Carlton, 1999). This stress may then be overcome once the ships dock, enabling fouling species to regain the energy needed for the next transoceanic voyage (Carlton, 1999). When a ship docks for even a short period of time, the fouling organisms may spawn and/or detach, leading to the colonization of the new environment (Minchin & Gollasch, 2003). If a vessel docks in a freshwater harbour, species such as oysters are able to close their shells and can survive for several days, whilst other sessile organisms may die off (Minchin & Gollasch, 2003).

In the north-western Mediterranean and Adriatic Sea, one of the most important vectors of alien species is mariculture. It has been estimated that this vector accounts for 78% of all introductions to the region (Galil, 2007). Alien species may be deliberately or accidentally introduced into an area through mariculture activities. In the first instance the target species is introduced for culture purposes. Such an

example can be seen in the Japanese oyster *Crassostrea gigas,* which was introduced into South Africa and France for aquaculture purposes (Grizel & Heral, 1991; Robinson *et al*., 2005b; Mead *et al*., 2011a). Besides initial introduction, target species may also experience intraregional transfer as they are moved within the boundaries of a country. The movement of the Mediterranean mussel *Mytilus galloprovincialis* from Saldanha Bay to Port Elizabeth is an example of such transfer (Branch & Steffani, 2004). The unintentional introduction of species occurs when these are associated with target species and inadvertently introduced along with the culture species. One such example is the sabellid worm *Terebrasabella heterouncinata* that was introduced into abalone farms in California via the import of abalone (*Haliotis midae*) from South Africa (Culver & Kuris, 2000). This invasive species was fortunately eradicated from the Californian farms, although this is an unusual achievement (Culver & Kuris, 2000).

Ecological impacts of invasive species

Invasive species have ecological impacts on native biota when they result in significant, measurable changes in the abundance of local species (Ruiz *et al*., 1999). In order to fully understand the impacts of an alien species on an area, the range, abundance and effect of the alien species on the environment need to be quantified (Parker *et al*., 1999). The impact of alien species in marine environments has received minimal attention, when compared to the impacts on terrestrial and freshwater systems (Ruiz *et al*., 1997; Grosholz *et al*., 2000), despite recognition that alien species pose a very serious threat to the biodiversity of marine ecosystems (Bax *et al*., 2001).

Invasive species affect recipient regions at five levels (Parker *et al*., 1999). Firstly, at the genetic level invasive species act through the alteration of natural selection pressures, caused by the invading species such as an alien predator (Parker *et al*., 1999). Secondly, the effect on individuals involves the impact of alien invaders on the growth and mortality of individuals (Parker *et al*., 1999). Thirdly there are population dynamic effects, which include impacts of invaders on the abundance and population growth of the indigenous species of an area (Parker *et al*., 1999). Such an example occurs along the Californian coast, where the green crab (*Carcinus maenas*) selectively preys upon particular local species (Grosholz *et al*., 2000). Since there is competition between some of these prey species, it was observed that due to the decrease in abundance of their competitor species, the species not preyed on as extensively, was increasing in number (Grosholz *et al*., 2000). Fourthly, at community level invasive species may cause an alteration of indigenous communities and their structure (Ruiz *et al*., 1997), as well as a decrease in biodiversity (McDonald, 2004; Bax *et al*., 2003). For example, the Mediterranean mussel *M. galloprovincialis* has become the dominant mussel along the South African west coast, altering the community structure of the rocky shore invertebrates in this region (Robinson *et al.,* 2007a). Lastly, invasive species can affect ecosystem processes (e.g. resource availability) (Lesser *et al*., 1992). An example of an impact at this level can be seen in the invading zebra mussel (*Dreissena polymorpha*), which removes plankton from the water column (Ruiz *et al*., 1997).

Alien species are themselves potential vectors for the introduction of new diseases and pests to an area (Ruiz *et al*., 1997; Bax *et al*., 2003; Ruesink *et al*., 2005; Haupt *et al*., 2010). This may impact native biota or human health. The Chinese mitten crab (*Eriocheir sinensis*) is one such example of this, in that it has invaded Europe and the US where it acts as an intermediate host of the human liver fluke (Bax *et al*., 2003).

Economic impacts of invasions

Any solid surface which is unprotected and exposed to a marine environment, eventually becomes fouled (Wahl, 1989). The impact caused by fouling species, becomes more serious the longer they are allowed to establish themselves (Bax *et al*., 2003). Fouling by marine species is the unwanted settlement of macro and microorganisms on man-made structures, resulting in the deterioration of these structures (Hellio, 2010). Negative economic impacts of invasive species relate to aquaculture, fisheries and fouling of marine infrastructure (Bax *et al*., 2003). The impact on economic production can result in negative social outcomes by reduced employment and a deterioration of the surrounding environment (Bax *et al*., 2003). Impacts such as these are seen in San Francisco Bay, where an invasion of the Asian clam (*Potamocorbula amurensis*) is thought to be the reason for the collapse of local fisheries (Bax *et al*., 2003). Furthermore the introduction of the North American ctenophore (*Mnemiopsis ledyi*) has resulted in the destruction of a $250 million fishery in the Black Sea (Ruiz *et al*., 1997).

Biofouling of aquaculture equipment by alien species is a constant problem, especially with the culture of oysters and scallops, which are grown in net cages (Lesser *et al*., 1992*;* Claereboudt *et al*., 1994; De Nys & Guenther, 2009). The fouling of aquaculture nets reduces the growth of the cultured species because the fouling species compete with the target species for food and reduce water flow through the nets (Wallace & Reisnes, 1985; Lesser *et al*., 1992; Claereboudt *et al*., 1994; Johnson *et al*., 2004; De Nys & Guenther, 2009). The frequent scraping of the fouling species off nets greatly increases the cost of aquaculture (Hodson *et al*., 1997).

Marine invasions in South Africa

By their very nature, alien species can have negative impacts on their receiving environment. Unfortunately, the impacts of only 5% of non-indigenous species occurring along the South Africa coast are known (Mead *et al*., 2011b). The most recent study considering alien marine species in South Africa, recorded 85 introduced and 39 cryptogenic (i.e. organisms whose true origin is unclear) marine and estuarine species, which accounts for 0.7% of all marine biodiversity in South Africa (Mead *et al.,* 2011b). Temporal analysis of the pattern of arrival of alien species in South African waters is hindered by an absence of routine monitoring of introductions into the country. Most invasions are therefore categorised according to date of first collection and not date of introduction (Mead *et al*., 2011b). Approximately 71% of the alien species present in South African waters were introduced via ship hull fouling (calculated from Mead *et al.,* 2011a).

The species which has been most successful in broadly invading the coast of South Africa is the mussel *M. galloprovincialis* (Robinson *et al*., 2005a; Robinson *et al.,* 2007a; Robinson *et al.,* 2007b; Branch *et al.,* 2008; Branch *et al.,* 2010). *M. galloprovincialis* dominates the South African west coast and extends as far east as East London (but at lower densities). Here this species alters the community composition of invaded shores (Robinson *et al.,* 2007a). This mussel displaces local species through competition, although its impacts are strongly moderated by wave action (Branch & Steffani, 2004; Rius & McQuaid, 2006; Branch *et al*., 2010). Despite negative impacts there have been positive economic implications arising from the *M. galloprovincialis* invasion. This mussel is now the sole target species of commercial mussel culture in South Africa (Stenton-Dozey *et al*., 1999; Robinson *et al.*, 2008).

Dominant invasive fouling species in Saldanha Bay

The Saldanha Bay system lies on the south west coast of South Africa and includes Langebaan Lagoon. It has a history as an important industrial node and the only deep water port on the west coast (Kruger *et al*., 2005). In addition, the West Coast National Park (the only marine protected area north of Cape Town) occurs within the Bay system (Weeks *et al*., 1991). There are a number of fish processing factories currently in operation in Saldanha Bay (Kruger *et al.*, 2005). Since the mid 1980's, Saldanha Bay has supported aquaculture operations which focus on the culture of oysters (*C. gigas*) and mussels (*M. galloprovincialis*) (Kruger, *et al.,* 2005). The high shipping volume and the presence of mariculture activities in Saldanha Bay, result in

it being vulnerable to the introduction of alien species via ballast water, hull fouling and mariculture.

Introduction of study species

The North Atlantic ascidian *Ciona intestinalis* has been recorded in North America, New Zealand, Australia, Korea, Hawaii, Chile and Hong Kong, although its native range is not clear (Blum *et al*., 2007; Zahn *et al*., 2010; Mead *et al*., 2011a, b). *C. intestinalis* strongly influences the succession of fouling communities along coast lines around the world (Lindeyer & Gittenberger, 2011; Sephton *et al*., 2011). It was first recorded in South Africa in 1955 and now occurs in sheltered bays along the coast of South Africa (Mead *et al.,* 2011a). The most likely vector responsible for the introduction of this species into South African waters is hull fouling (Mead *et al*., 2011a). This ascidian usually attaches to ropes, kelp and mussel or oyster rafts in sheltered bays and harbours (Mead *et al*., 2011a) and usually recruits onto poorly lit, downward facing surfaces (Howes *et al*., 2007; Rius *et al*., 2010). The fact that *C. intestinalis* individuals are easily removed when vessels move at speed (Millard, 1951), makes it an important fouling species only on ships which are docked for long periods of time (Millard, 1951).

C. intestinalis is a sessile, solitary marine hermaphroditic ascidian up to 15 cm long (Millard, 1951; Figure 1.1). The body is surrounded by a greenish, gelatinous tunic (McDonald, 2004). It is a broadcast spawner, with fertilisation occurring in the water column (Howes *et al.*, 2007; Therriault & Herborg, 2008; Zahn *et al*., 2010). Settlement occurs throughout the year but predominantly from March to June (autumn) in the southern hemisphere (Scheer, 1945; Millard, 1951). Temperature

probably plays an important role in the recruitment time of this species (Howes *et al.,* 2007). *C. intestinalis* has an average lifespan of six months (Millard, 1951), with a maximum lifespan of two years (Blum *et al*., 2007). It is thought to be photosensitive, recruiting at depths between 4.5 m and 8.5 m (Kajiwara & Yoshida, 1985; Howes *et al*., 2007). In Table Bay Harbour, Cape Town, *C. intestinalis* displaced slower growing barnacles, but only on the underside of settlement plates, likely reflecting this species sensitivity to light (Millard, 1951). *C. intestinalis* produces antimicrobial compounds and mucus which inhibit epibiosis, thus excluding competition from other organisms (Davis, 1998).

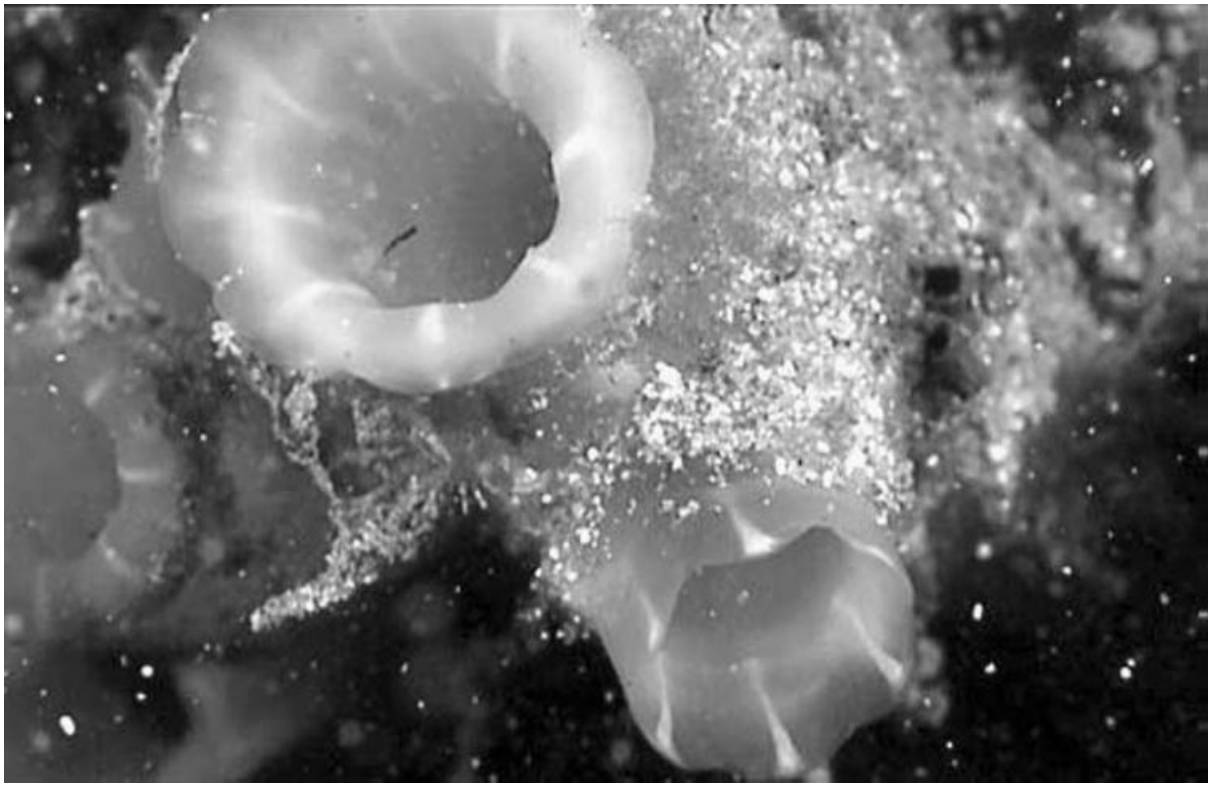

Figure 1.1: *Ciona intestinalis*. (McDonald, 2004)

C. intestinalis has become a nuisance fouling organism for numerous shellfish aquaculture ventures worldwide (Howes *et al.,* 2007; Blum *et al*., 2007; Edwards & Leung, 2009; Ramsay *et al.*, 2009). In northern Chile, *C. intestinalis* is a pest in bivalve aquaculture facilities, as it attaches to culture ropes, out-competing target species for space (Uribe & Etchepare, 2002; Castilla *et al*., 2005). In South Africa, the fouling by this species on mussel stocks increases labour expenses (Carver *et al*., 2006). Despite its large geographical distribution, the ecological impacts of *C. intestinalis* have only been considered in San Francisco Bay (Blum *et al*., 2007) where it has been found to decrease species richness in native fouling communities (Blum *et al*., 2007).

The ecological impacts of *C. intestinalis* invasion in South Africa have not been studied, although it has been suggested that this species has negative effects on the mussel and oyster farms of Saldanha Bay (Mead *et al*., 2011b). Economic losses due to the removal of this species from mussel farms in Saldanha Bay have been reported to be R100 000 per annum (Robinson *et al*., 2005a).

Against this backdrop, this project aims to 1) assess the impact of *C. intestinalis* on indigenous fouling communities in Saldanha Bay, 2) quantify the prevalence of alien fouling species associated with oyster culture in the Bay and 3) assess the impact of *C. intestinalis* on the survival, growth and condition of cultured oysters.

Chapter 2

The impact of the alien ascidian *Ciona intestinalis* on fouling communities of Saldanha Bay

Abstract

Ascidians form part of marine fouling communities around the world. Often these communities include alien species that may affect native fouling biota. This chapter had two aims, firstly to investigate the impact of the alien ascidian *Ciona intestinalis* on indigenous fouling communities and secondly to assess the role of water movement and depth in moderating the impact of this species in Saldanha Bay. The impacts of *C. intestinalis* on community structure and diversity on perspex settlement plates were quantified under high (mean flow rate: 1 m/sec) and low water (mean flow rate 0.12 m/sec) movement conditions, at two depths (0.6 m and 3.1 m). *C. intestinalis* was removed from treatment plates every two weeks, while control plates were left undisturbed for the full 16 weeks. Treatment control plates were removed from the water for the same time it took to remove the *C. intestinalis* individuals from the treatment plates. Unexpectedly, *C. intestinalis* settled only on deep plates and under sheltered conditions, where it showed no significant impact on community composition or the diversity of fouling communities. This unanticipated result may be due to high spatial variability in settlement of *C. intestinalis*, or low settlement densities of this species recorded in this once-off study.

2.1 Introduction

Alien invasive species are well recognised as threats to their receiving environments (Molnar *et al*., 2008; Needles & Wendt, 2013). The most severe environmental consequences of invasions include the displacement of indigenous species (Branch & Steffani, 2004; Molnar *et al*., 2008), alteration of existing community structures (Pimentel *et al*., 2005; Robinson *et al*., 2007a) and the reduction of species richness (Altman & Whitlatch, 2007; Blum *et al*., 2007). Alien ascidians are common in coastal fouling communities (Lambert, 2002; Locke & Carman, 2009), where they rapidly colonise marine structures (Lambert, 2007). This is likely due to many ascidians having wide environmental tolerances, especially with regard to temperature and salinity (Sims, 1984; Nomaguchi *et al.,* 1997; Therriault & Herborg, 2008). These characteristics allow them to successfully invade a variety of marine environments (Lambert, 2002). The relatively poor natural dispersal ability of ascidians (Petersen & Svane, 1995; Lambert, 2005) means that their spread to foreign regions is dependent on human-mediated transfer, and it has consequently been suggested that ascidians could be used as bio-invasion indicators (Marins *et al*., 2010). The dominant vector of introduced ascidians is thought to be hull fouling (Wasson *et al*, 2001; Lambert & Lambert, 2003).

The development of fouling communities can be influenced by alien species that control the resources available to indigenous biota (Jones *et al*., 1994; Wright & Jones, 2006; Lutz-Collins, *et al*. 2009). Sessile alien species, such as ascidians can dramatically alter their receiving environments by functioning as environmental engineers (Castilla *et al*., 2004; Silliman & Bertness, 2004; Dijkstra *et al*., 2007).

Ecosystem engineers take two forms, autogenic and allogenic (Jones *et al*., 1994). Autogenic engineers modify their habitats via their presence or physical structure (Jones *et al*., 1994). Such organisms include mussels and oysters which filter nutrients from the water column and provide artificial habitats in the form of their shells (Alagarswami & Chellam, 1976). In contrast, allogenic engineers modify their habitats via their activities, by changing living or non-living materials from one physical state to another (Jones *et al*., 1994; Coleman & Williams, 2002). Introduced colonial ascidians can act as allogenic engineers when they form dense mats along pebble beaches, smothering indigenous biota and altering indigenous communities (Bullard *et al*., 2007; Mercer *et al*., 2009). Additionally, non-colonial species have been found to alter species composition and richness by outcompeting indigenous epi-macrofauna for space and nutrients (Blum *et al*., 2007; Lengyel *et al*., 2009; Daigle & Herbinger, 2009). Often the alterations caused to the environment by alien species facilitate further invasions, resulting in a cascade of shifts in community structure (Castilla *et al*., 2004; Mercer *et al*., 2009).

In South Africa nine introduced ascidians have been recorded (Mead et al. 2011a) with one of the most wide spread and common being *Ciona intestinalis* (Rius *et al*., 2011). This species was first reported from Durban (Millar, 1955) and is now present along the whole coast, where it attaches to kelp, mussel rafts and harbour ropes in sheltered areas (Mead *et al*., 2011a; Rius *et al*., 2011).

Water movement is well known to influence the structure of sessile marine communities (Smith, 1946; Loya, 1976; Cowen *et al*., 1982; Bulleri & Airoldi, 2005;

Branch *et al*., 2010). This impact can manifest through physical removal of individuals (Herman & Smith, 1951; Cheshire & Collings, 1999), displacement of nutrients (Sebens & Johnson, 1992; Blamey & Branch, 2008) and alterations in growth rates (Kirby-Smith, 1972; Steffani & Branch, 2003). Water movement is a powerful force which impacts both individuals and communities (Vogel, 1984; Kaandorp, 1999). As with numerous other sessile species, the distribution of ascidians is influenced by water movement (Hernandez-Zanuy & Carballo, 2001). Hernandez-Zanuy & Carballo (2001) and Lambert & Lambert (2003) found that ascidians display one of three distinct settlement patterns, i.e. settlement associated with only high water movement habitats, settlement associated with more sheltered areas and settlement with no preference for or avoidance of certain exposure levels.. The characteristic tendency of *C. intestinalis* to recruit in sheltered areas (Rius *et al*., 2011) suggests that water movement may be a significant moderator of this species recruitment and hence any impact it may have on fouling communities.

Despite its wide distribution, the ecological impact of *C. intesinalis* remains un-quantified in South Africa. This study aimed to assess the impact of this alien tunicate on fouling community composition and diversity. Two *a priori* hypotheses were tested in this chapter: (1) The presence of *C. intestinalis* would decrease species richness and alter community composition. (2) These impacts would be moderated by water movement, with sheltered areas supporting more *C. intestinalis* and experiencing greater influence by this invasive species than areas exposed to higher water movement. These hypotheses were tested at two depths.

2.2 Methods

Study site

The Saldanha Bay system lies on the south west coast of South Africa (approximately 100 km north of Cape Town) and includes Langebaan Lagoon. It is the only sheltered deep water port north of Cape Town, and serves as an industrial node for import and export via shipping (Weeks *et al*., 1991; Kruger *et al*., 2005). In addition, the West Coast National Park, the only marine protected area north of Cape Town, lies adjacent to Saldanha Bay (Weeks *et al*., 1991). Saldanha Bay is subdivided into Big Bay (south of the iron ore jetty, Figure 2.1) and Small Bay (north of the iron ore jetty) (Weeks *et al*., 1991). Water circulation within both bays is predominately wind-driven, whilst the currents in Langebaan Lagoon are predominately influenced by tidal movements (Weeks *et al*., 1991; Monteiro & Largier, 1999).

This study took place at two sites, Yacht Port Marina (33º13'S, 17°57'E) and Langebaan Yacht Club (33º54'S, 18°27'E). The marina is within breakwaters and in the lee of the causeway linking Marcus Island and the mainland (Figure 2.1) and consequently is very sheltered, with minimal wave action and weak currents. Before construction of the iron ore jetty, surface current velocities in this area were less than 0.12 m/sec (Shannon & Stander 1977). Since then, additional construction of the breakwaters around Yacht Port Marina to protect the moored tug boats and yachts is likely to have reduced current velocities further. In contrast, Langebaan Yacht Club is on the eastern shore of the entrance to Langebaan Lagoon, and experiences strong tidal currents of 1 m/sec (Shannon & Stander, 1977).

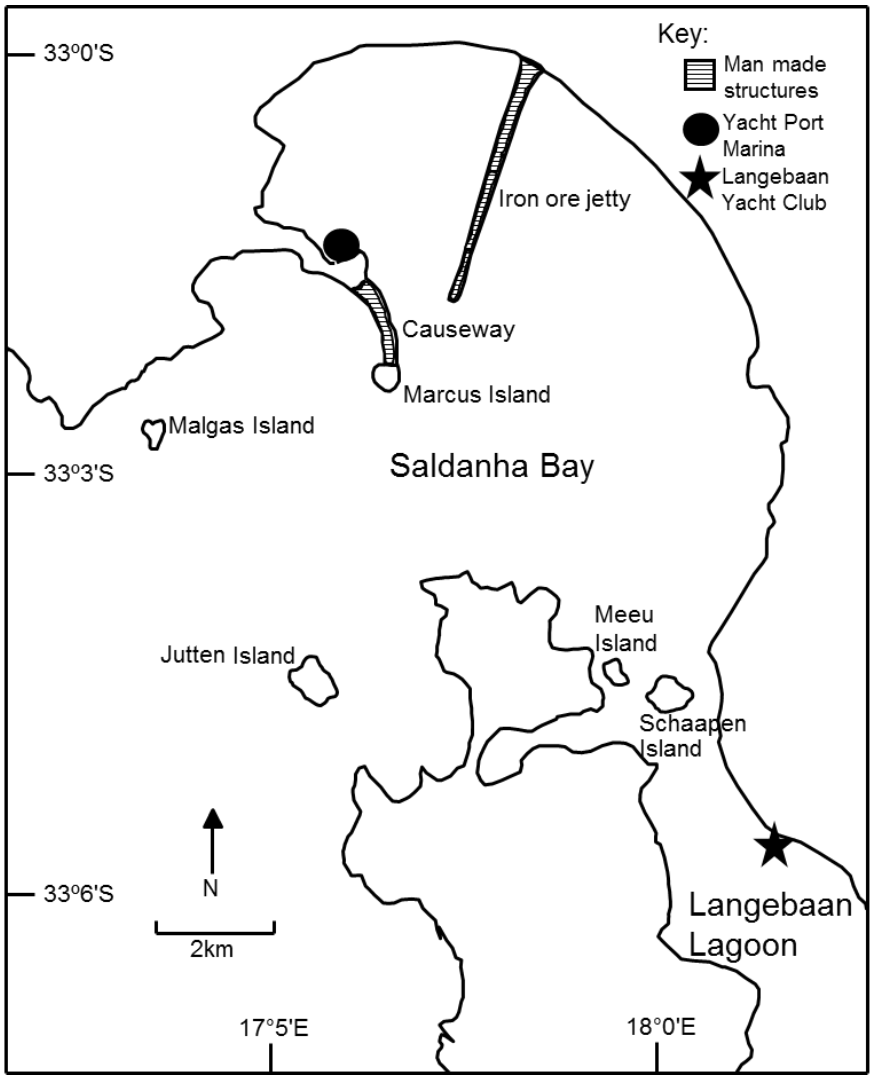

Figure 2.1: Site 1, Yacht Port Marina (●) and Site 2, Langebaan Yacht Club (★).

Experimental design

To assess the impact of *Ciona intestinalis* on indigenous fouling communities, 18 arrays, each comprising two opaque perspex plates (20x20 cm, Figure 2.2) were deployed at each site. The upper and lower surfaces were lightly sanded before deployment (Blum *et al*. 2007). These plates were suspended in the water column

from cleats on walk-on jetties to depths of 0.6 m (hereafter referred to as shallow plates) and 3.1 m (here after referred to as deep plates, Figure 2.2). Ropes were deployed between 2.6 and 7.7 m apart, this spacing was determined by available cleats on the jetties.

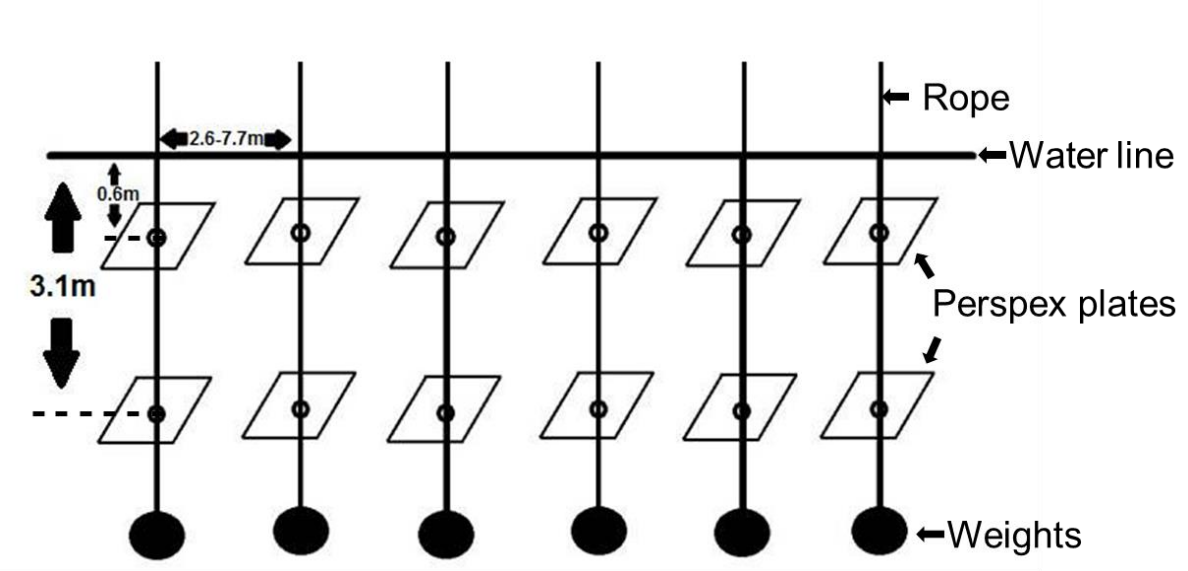

Figure 2.2: Configuration of perspex plates deployed to assess the impact of *Ciona intestinalis* on indigenous fouling communities, as well as the moderating effect of water movement on the impact of this species.

Three treatments were set up: (1) *Ciona* removal treatment plates that were subjected to the removal of all *C. intestinalis* by hand, every two weeks; (2) treatment control plates that were removed from the water for the same length of time as the treatment plates, but remained otherwise untouched; (3) control plates that were left undisturbed for the entire experiment. Six replicate plates were deployed per treatment per depth. Plates were deployed in June 2012 and the experiment was run

for 16 weeks. This period includes the main settlement season of *C. intestinalis,* which occurs during austral winter (Scheer, 1945; Millard, 1951). At the completion of the experiment all plates were photographed before biota were removed and preserved for later identification to the lowest possible taxonomic level. These photographs were used to aid in the later identification of organisms. For all processing and analysis, the top and bottom surfaces of the plates were treated separately. Following identification, biota were counted and wet weighed.

Statistics analysis

Analysis of community structure was performed using the Primer-6 software package and was based on non-standardized, fourth-root transformed wet biomass data. An ANOSIM was used to detect differences in community structure between the three treatment groups. Multidimensional scaling (MDS) plots and cluster diagrams were used to visually illustrate the relationships between treatments. Diversity was compared between treatments using the Shannon Wiener, Pielou's evenness and Margalefs indices.

The Shannon Wiener diversity index (*H'*) incorporates components of both species richness and equality and is a measure of diversity (Clarke & Warwick, 1994). This index is given by the equation:

$$H' = -\sum_i p_i (\log p_i)$$

Where p_i is the proportion of the total number of individuals arising from the *i*th species.

The Margalef's index (*d*) measures species richness, using the following equation:

$$d = \frac{S - 1}{\log N}$$

Where *S* is the total number of species and *N* is the total number of individuals.

Equitability is usually expressed as Pielou's evenness index, which estimates how evenly individuals are distributed among species. The Pielou's evenness index (*J'*) is calculated using the equation:

$$J' = H'(observed) / H'_{max}$$

Where H'_{max} is the maximum possible diversity, which would be achieved if all species were equally abundant (= *log S*).

Following consideration of normality (Shapiro-Wilks normality test) and variances (Levene's test) measures of biomass (g/m^2), densities (kg/m^2) and diversity indices were compared between treatments, using Kruskal Wallis ANOVAs. Kruskal Wallis ANOVAs were used to test for significant differences in both the abundance (biomass and density) of alien and indigenous species between the three treatment groups. The statistical package STATISTICA was used to perform all univariate statistical tests.

2.3 Results

During the experiment, 16 of the original 36 plates were lost at the high water movement site, Langebaan Yacht Club, probably because of strong tidal flow. No

plates were lost under low water movement conditions at Yacht Port Marina. No settlement of *Ciona intestinalis* was recorded on any experimental plates at the high water movement site, and only a single shallow plate had *C. intestinalis* recruitment. *C. intestinalis* settled predominately on the deep plates under the sheltered conditions of the marina (Figure 2.3), with total numbers of recruits varying from zero to 72 individuals per plate and biomass ranging from zero to 24.1g per plate.

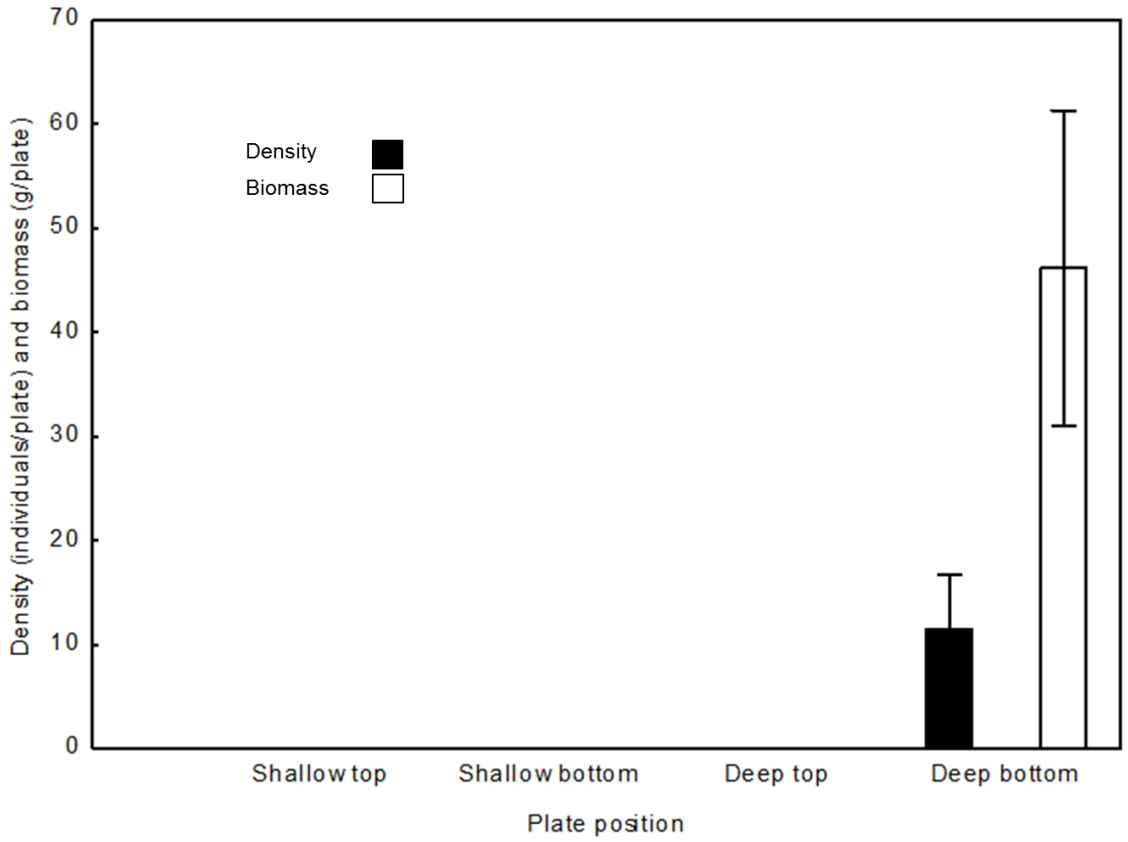

Figure 2.3: Mean density (individuals/plate ± 1 SE) and biomass (g/plate ± 1 SE) of *C. intestinalis* on treatment plates at the end of the experiment at the low water movement site. No *C. intestinalis* were recorded at the high water movement site. Total area for each plate was 400 cm^2.

The absence of *C. intestinalis* settlement at Langebaan Yacht Club and the fact that only one shallow plate at Yacht Port Marina had recruits precluded statistical comparisons between low and high water movement conditions, or different depths. Under low water movement conditions *C. intestinalis* had no impact on the structure of fouling communities (ANOSIM, R = 0.038, p > 0.05) (Figure 2.4) nor on diversity, regardless of the diversity measure employed (Kruskal-Wallis ANOVA, p > 0.05 in all cases) (Figure 2.5).

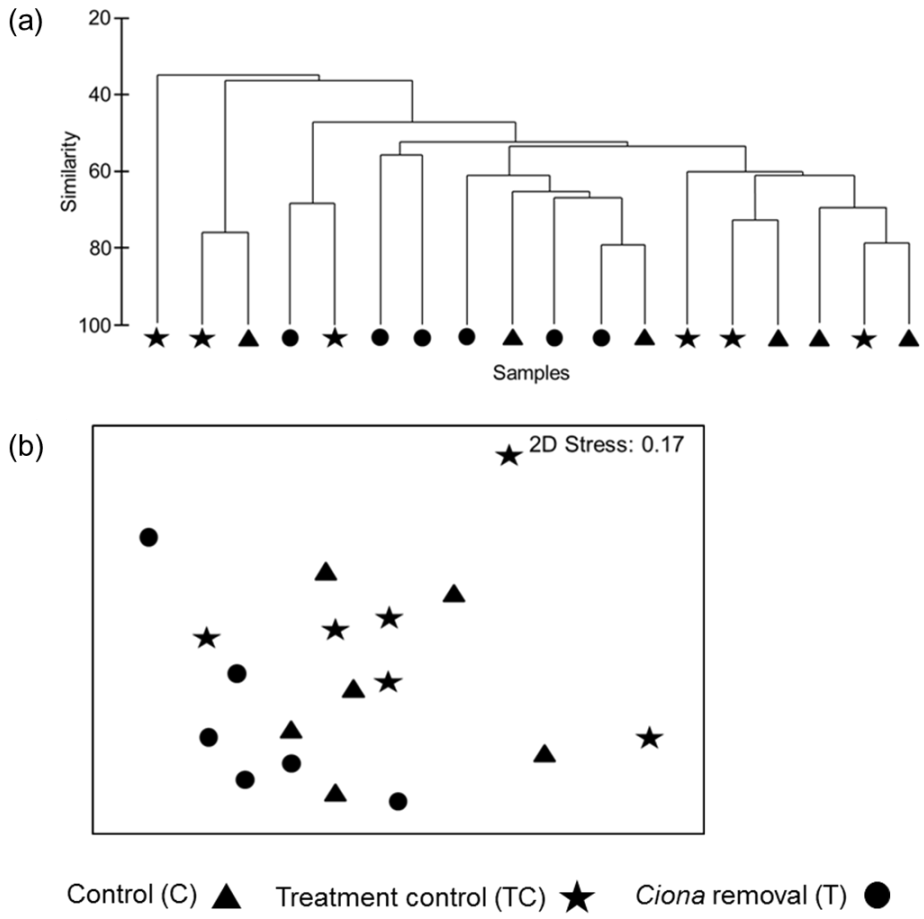

Figure 2.4: (a) Non-metric multidimensional scaling (MDS) and (b) CLUSTER plots of species biomass per plate present on deep bottom plates deployed at Yacht Port Marina.

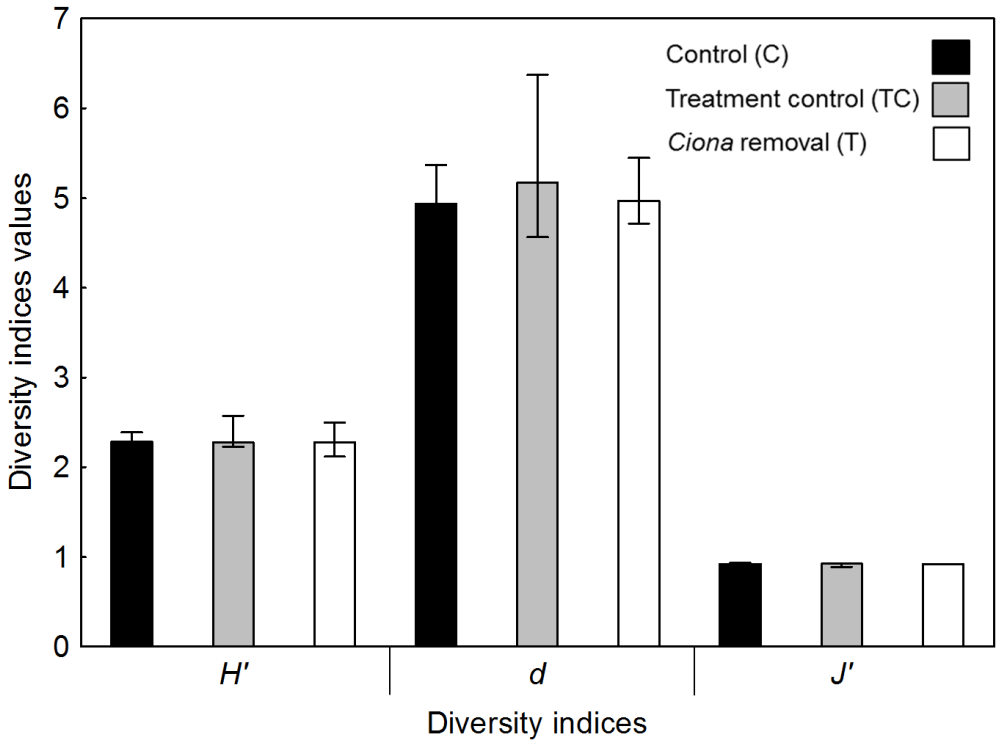

Figure 2.5: Median (± 25%-75% percentiles) Shannon Wiener (H'), Margalef's (d) and Pielou's evenness (J') diversity indices for biomass (g/m^2) of *Ciona* removal, treatment control and control plates recorded on deep plates under sheltered conditions in Yacht Port Marina.

No significant differences were recorded between the biomass or density supported by alien (Kruskal-Wallis ANOVA, $p > 0.05$ in all cases) and indigenous (Kruskal-Wallis ANOVA, $p > 0.05$ in all cases) species in the three treatments (Figure 2.6).

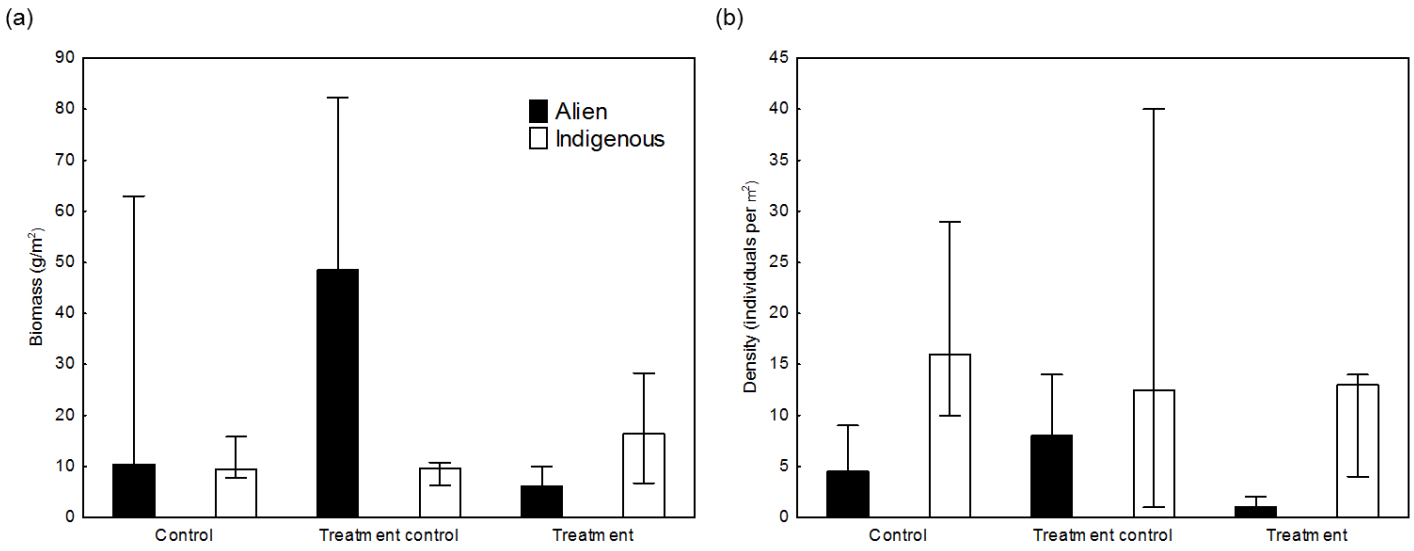

Figure 2.6: Median (a) biomass (g/m^2) (25%-75% percentiles) and (b) density (individuals/m^2) (25%-75% percentiles) of alien and indigenous species on the undersides of deep plates at Yacht Port Marina.

A total of 51 species were found on the three treatment group plates at the end of the experiment. Of these, 11 were alien, two were cryptogenic and 30 were indigenous. Eight organisms from the following taxa could not be identified to species level: Decapoda (crayfish), Polychaete (*Spirorbis*), Nematode, Hydozoa (*Eudendrium*) and Chlorophyta (*Ulva*, *Rhizoclonium*) could not be identified to species level (Appendix 2.1).

2.4 Discussion

This study showed that in Saldanha Bay, both settlement and recruitment of the invasive ascidian *Ciona intestinalis* were moderated by water movement, and that this species had no impact on species richness under the experimental conditions

we created. In other countries overseas, this species maintains dominant growth over other fouling species (Carver *et al*., 2003), including other ascidians (Ramsay *et al*., 2008). It has been found to decrease species richness and change fouling community composition in San Francisco Bay (Blum *et al*., 2007).

C. intestinalis recruits settled predominately on the undersides of deep plates under the sheltered conditions of Yacht Port Marina. The lack of settlement by this species at Langebaan Yacht Club is likely a reflection of the high water movement at this site. This finding aligns with previous observations that this species prefers sheltered conditions (Howes *et al*., 2007; Mead et al 2011a). The low numbers of recruits on the upper surface of the experimental plates was expected, as Howes *et al*. (2007) found that *C. intestinalis* settled mostly on the undersides, rather than the upper surface of submerged structures. This characteristic settlement pattern results as larvae become photosensitive, sinking deeper down the water column, where there is lower light intensity (Kajiwara & Yoshida, 1985). As a result, settlement tends to occur low in the water column and in shaded areas. An interesting observation of this study was the fouling of *C. intestinalis* individuals. Despite the fact that this species is known to produce antifouling mucus (Davis, 1998), both the indigenous barnacle *Notomegabalanus algicola* and the alien colonial ascidian *Botryllus schlosseri* were found attached to *C. intestinalis*.

It was anticipated that the presence of *C. intestinalis* would reduce species richness and alter community composition (Blum *et al.*, 2007). However in this study, diversity was unaffected by the presence of this alien ascidian. While this finding was

unexpected, it may be explained by two factors. Firstly, mean 135 ± 91 SE recruit densities recorded in this study were roughly 1% of those recorded by Blum *et al*. (2007) and relatively low when compared to those recorded by Carver *et al*. (2003), Howes *et al*., (2007) and Rius *et al*. (2011) (Table 2.1). The different methodologies used during these experiments need to be taken into consideration, however the density of recruits recorded during this study are remarkably lower than other studies. At these densities *C. intestinalis* may be at abundances too low to influence the fouling community studied. Secondly, *C. intestinalis* did not settle uniformly across all treatment plates as indicated by the high variability in the number of recruits removed from the plates. This high variability in settlement was not expected and may have obscured community-level impacts (Blum *et al*., 2007). A study conducted by Rius *et al*. (2011) in Saldanha Bay, found that the density of *C. intestinalis* recorded on mussel ropes within Small Bay had decreased in 2010, when compared to 1994. This may indicate a decrease in the abundance of *C. intestinalis* in Saldanha Bay, or inter-annual variability in the spawning of this species. This species has been noted to have highly irregular recruitment peaks, not linked to water temperature (Keough, 1983).

Table 2.1: Comparison of total number of *Ciona intestinalis* recorded during experiments conducted by several authors in different locations.

Average number of *Ciona intestinalis* (per m^2) ± (SE) recorded	Time of sample	Running time of experiment	Water movement where samples were recorded	Substrate	Location	Outcome of experiment	Citation
1190 ± 349	December 1993 to May 1994	Six months	Low, sheltered bay	Suspended mussel ropes	Saldanha Bay, South Africa	Numbers of *C. intestinalis* decrease between samples recoded in 1994 and 2010	Rius *et al*. (2011)
360 ± 121	December 2009 to March 2010	Four months	Low, sheltered bay	Suspended mussel ropes	Saldanha Bay, South Africa	Same as above	Rius *et al*. (2011)
1321 ± 1093	March 2002 to March 2003	One year	Low, study took place in a harbour	Suspended pates	Richmond Marina Bay Yacht Harbour, San Francisco Bay	Species richness is negatively correlated to *C. intestinalis* abundance	Blum *et al*. (2007)
5018 ± 1538	May 2003 to December 2003	Six months (2003)	Low (average 0.3 cm/s)	Suspended pates	Indian Point, Nova Scotia	*C. intestinalis* settled predominately at a depth of 4.5m	Howes *et al*. (2007)
1604 ± 470	June 2004 to December 2004	Six months (2004)	Low (average 0.3 cm/s)	Suspended pates	Indian Point, Nova Scotia	As above	Howes *et al*. (2007)
209 ± 76	April 2000 to September 2000	Six months	No details given	Suspended mesh plates	Lunenburg Bay, Nova Scotia	Acetic acid is more effective at eliminating *C. intestinalis* than hydrated lime, saturated brine or hypochlorite solution	Carver *et al*. (2003)

It would be beneficial to reproduce this study over a number of years and during each of the four seasons. It would also be advantageous to assess the settlement patterns and impacts on fouling communities by this species in various sheltered areas along the South African coastline, to better understand its impact on fouling communities in South Africa.

In San Francisco Bay, *C. intestinalis* has been found to reduce species richness (Blum *et al*., 2007), but this was not the case under the experimental conditions of the present study. No *C. intestinalis* were recorded at the high water movement site at Langebaan Yacht Club, in Langebaan Lagoon. Furthermore, this species was recorded predominantly on the underside of the deep plates placed in Yacht Port Marina. The presence of invasive species within fouling communities raises conservational concerns and monitoring may need to be developed in order to measure long term changes in the ratio of invasive to indigenous fouling species.

Chapter 3

The prevalence of alien fouling species affecting Saldanha Bay oyster farms

Abstract

Temporal changes in the composition and diversity of fouling communities occur under the influence of a number of environmental and ecological factors. These fouling communities may contain introduced alien species, which have the potential to negatively impact the indigenous species. Monitoring the composition of fouling communities is necessary to determine the frequency of introductions and abundance of alien species in a habitat. This experiment aimed to document seasonal trends in the fouling communities that affect oyster farms in Saldanha Bay, and assess the prevalence of alien species in these communities. Fouling samples were collected using 20x20 cm scrape quadrats from the upper and lower sides of the shallow (1.5 m depth) and deep (2.9 m depth) oyster cages in Big Bay during January, April, July and October (2013). Results showed that community structure differed significantly between seasons and depth. Alien species were recorded in higher abundances than indigenous species. The high biomass of filter feeding alien fouling species recorded may have negative impacts on the growth of the cultured oysters. Further studies are required to monitor settlement preferences and annual abundances of alien species.

3.1 Introduction

Vectors associated with shipping and aquaculture operations are an important introduction pathway for both target species (Naylor *et al*., 2001; Branch & Steffani, 2004) and associated organisms (Bax *et al*., 2003; Haupt *et al*., 2010). In South Africa, the Japanese oyster *Crassostrea gigas* was imported by the aquaculture industry (Robinson *et al*., 2005a), but has subsequently formed naturalized populations in estuaries along the South African coast (Robinson *et al*., 2005b). In the same way pathogens and parasites associated with aquaculture species can be unintentionally introduced during the movement of stock (Culver & Kuris, 2000; Naylor *et al*., 2001; Weigle *et al*., 2005; Streftaris & Zenetos, 2006; McKindsey *et al*., 2007). The nets and cages used for oyster mariculture can provide artificial habitats for fouling species (Hodson *et al*., 2000; Dealteris *et al*., 2004; Ross *et al*., 2004), by providing substrate for recruitment (Bulleri & Airoldi, 2005). In some cases, artificial structures have been found to have more alien fouling species attached to them than the neighbouring rocky reefs (Glasby *et al*., 2007). In the same way as infrastructure, target species also themselves act as habitat to alien fouling species both those that burrow into their shells and attach to the shell surface (Alagarswami & Chellam, 1976; Ross *et al*., 2004).

Alien fouling species negatively affect commercially important species such as those cultured in aquaculture facilities (Altman & Whitlatch, 2007). The growth of fouling communities on aquaculture cages is often rapid, driven by the organically rich waste formed from uneaten food and faecal matter from the cultured organisms (De Nys & Guenther, 2009). Biofouling of aquaculture cages and nets reduces water flow

through the farming system (Costelloe *et al*., 1996; Ross *et al*., 2002). This leads to a reduction in oxygen supply and particulate organic material (Wallace & Reisnes, 1985) and a build-up of metabolic ammonia (De Nys & Guenther, 2009) that may be harmful to the cultured organisms (De Nys & Guenther, 2009). The accumulation of fouling organisms on aquaculture cages can also reduce the buoyancy (Gittenberger, 2009) and negatively affect the structural integrity of culture infrastructure (De Nys & Guenther, 2009). Together these impacts can result in reduced growth of the target species and diminished economic profits (Wallace & Reisnes, 1985; Lesser *et al*., 1992; Claereboudt *et al*., 1994; Johnson *et al*., 2004).

The potential of introductions to Saldanha Bay is particularly concerning, as the West Coast National Park (the only marine protected area north of Cape Town), lies adjacent to Saldanha Bay. Saldanha Bay is made vulnerable to introductions of alien species by the high shipping volume experienced and the several mariculture operations located within Small and Big Bays. This study aims to document seasonal trends in the fouling communities that affect oyster farms in Saldanha Bay, and assess the prevalence of alien species in these communities. This is of particular interest as oyster farms may be a source of alien species introductions but can also be negatively affected by them.

3.2 Methods

Study site

This study was undertaken at the Saldanha Bay Oyster Company that farms the Pacific oyster *Crassostrea gigas*. This farm uses suspended culture on a long line system in both Small and Big bays. This study used the lines situated in Big Bay (Figure 3.1).

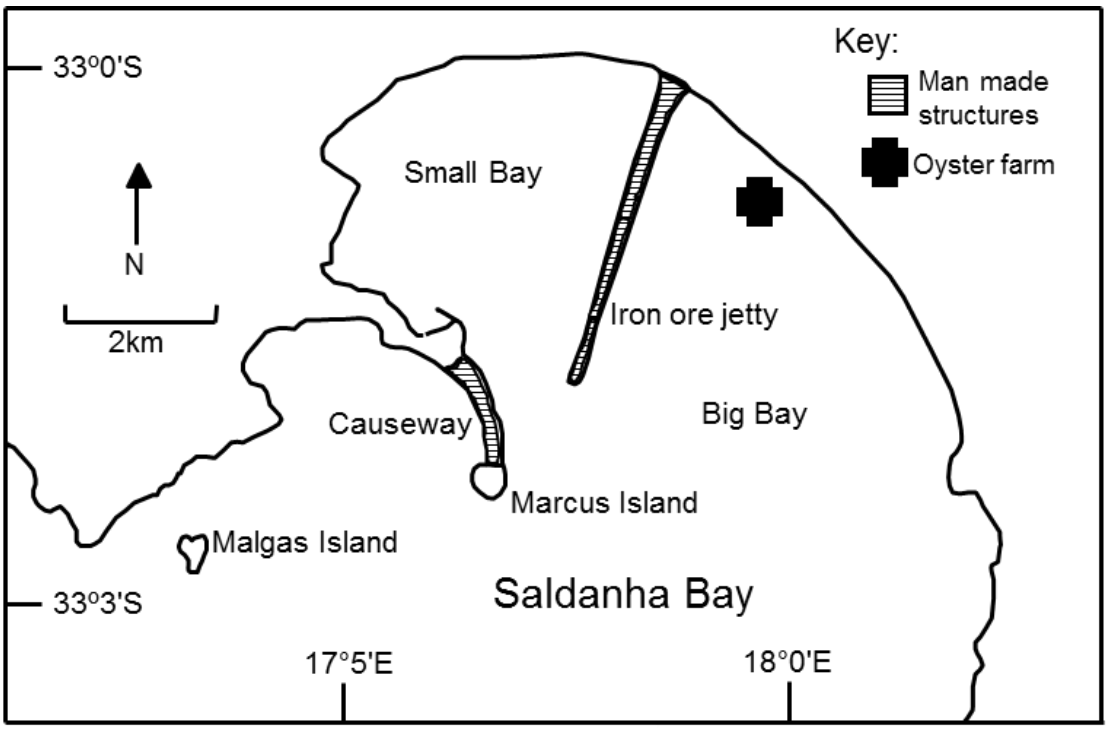

Figure 3.1: Saldanha Bay with position of the oyster farm, Saldanha Bay Oyster Company, in Big Bay.

This farming system comprises 200 m horizontal lines, with anchor lines at each end attached to mooring blocks on the sea bed, and to large end floats on the surface. Each line holds roughly 150 “stacks” comprising five high-density polyethylene cages (strung together in a vertical “stack”, Figure 3.2), with small barrel floats placed in

between cages. Stacks are suspended from the long-line. During the culture process oysters remain submerged, and are removed from the water every two months for cleaning, sorting according to weight, and either replanting in clean cages or shipping to market (A.F.G. Tonin *pers. comm*.).

Sampling design

In order to assess seasonal trends in species composition as well as density (individuals/m^2) and biomass (g/m^2) of invertebrates within fouling communities, samples were collected from oyster stacks in summer (January), autumn (April), winter (July) and spring (October) of 2012. Stacks were sampled after spending a period of two months in the water to align with normal farming operations. At each sampling time, samples were collected from six randomly-selected oyster stacks. For each stack one 20x20 cm scrape quadrat was randomly taken from both the upper and lower sides of the shallow and deep cages (Figure 3.2). Thus four samples were collected per stack, each stratified by depth and orientation. While quadrats were randomly placed, the outer 10 cm of the cages were avoided so as to avoid the edge effect (Gundersen, 1977). Shallow cages were fixed at between 1 and 1.5 m below the sea surface, and deep cages at between 2.4 and 2.9 m. Following collection, samples were identified to the lowest taxonomic level possible. Unitary individuals were counted and weighed, while algae and colonial organisms were weighed only. Samples were weighed to the nearest gram.

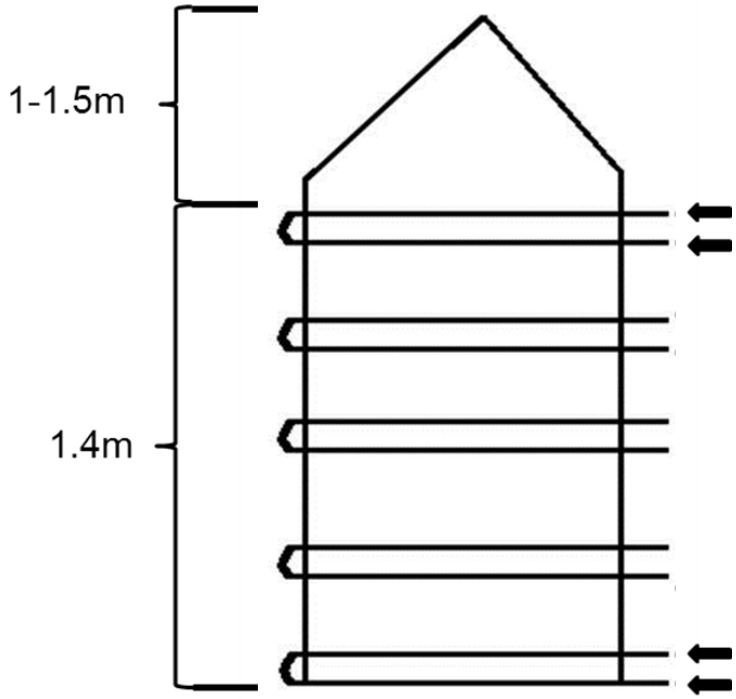

Figure 3.2: An oyster stack showing where scrape samples were collected.

Statistical analysis

All multivariate analyses were conducted using the Primer-6 software package. Due to the presence of colonial species all community analyses were conducted using biomass. Community structure, based on non-standardized, fourth-root transformed wet biomass (g/m^2) data, was compared between depths, orientation and sampling periods using PERMANOVA. SIMPER was used to identify which species most influenced differences between communities. Hierarchical cluster analysis and non-metric MDS plots were used to produce graphic representations of the relationships between the samples.

The statistical package R was used to construct Generalised Least Squares models (GLS) to assess: 1) the combined effect of season (summer, autumn, winter and

spring), depth (deep and shallow) and orientation (top and bottom) on diversity (as measured by the Shannon Wiener index and Pielou's evenness index); 2) total biomass (g/m^2) and density ($individuals/m^2$) of fouling organisms; 3) biomass (g/m^2) and density ($individuals/m^2$) of alien and indigenous species separately; and 4) that of the species identified by SIMPER as contributing to differences between communities. The best fit model for each dependent variable was selected, based on Akaike Information Criterion values. ANOVAs were conducted in R to assess the individual effect of season, depth and orientation on the dependent variables.

3.3 Results

PERMANOVA showed that both season and depth had a significant effect on community structure (Table 3.1, Figure 3.3). SIMPER analysis showed that four alien species most influenced the differences observed in community composition. These were the mussels *Mytilus galloprovincialis* and *Semimytilus algosus*, the amphipod *Jassa marmorata* and the ascidian *Ciona intestinalis*.

Table 3.1: Results of a multifactorial PERMANOVA on the effects of season, depth and orientation on fouling community composition. ns = non-significant results ($p > 0.05$).

Source	df	MS	*F*	*p*
Season	3	15042	63.444	$p < 0.05$
Orientation	1	122.86	0.51819	ns
Depth	1	2703	11.4	$p < 0.05$
Season x Orientation	3	429.76	1.8126	$p < 0.05$
Season x Depth	3	1215.5	5.1269	$p < 0.05$
Orientation x Depth	1	193.72	0.81706	ns
Season x Orientation x Depth	3	324.55	1.3689	ns

A GLS model found that together season, depth and orientation significantly affected the Shannon Wiener index (*H'*). However, the ANOVA considering the individual contributions of these factors showed season to be the only factor to have a significant effect (Table 3.2, Figure 3.4), with significantly higher diversity recorded in autumn and winter (Table 1, Figure 3.4, Appendix 3.1). In contrast, Pielou's evenness index (*J'*) was found to be best predicted only by season and depth (best fit GLS model). An ANOVA showed that both these factors had significant effects on Pielou's evenness index when considered independently (Table 3.2), with highest evenness being recorded on shallow cages in autumn (Table 1, Figure 3.4, Appendix 3.1).

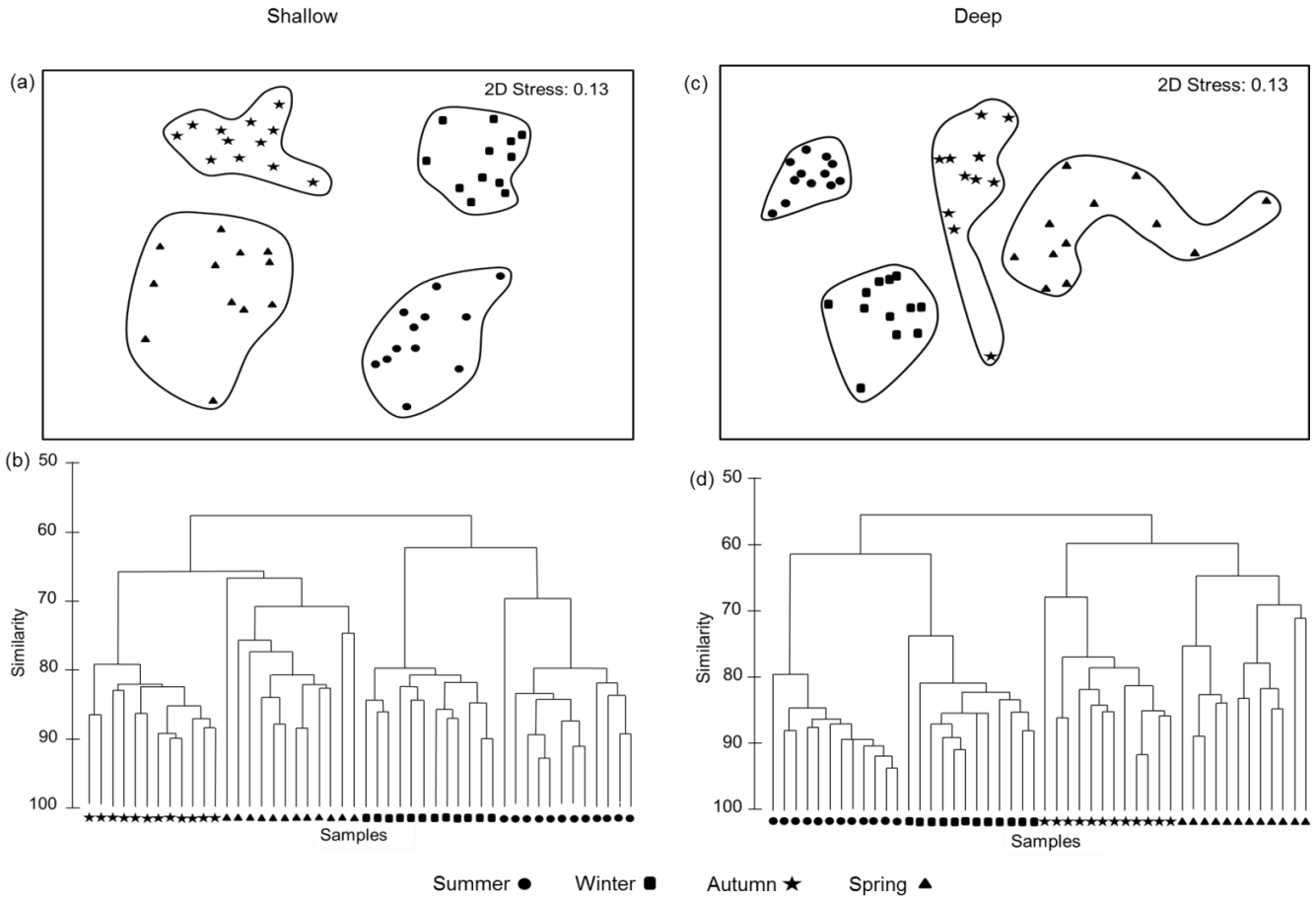

Figure 3.3: Cluster and MDS plots based on species biomass (g/m^2) for samples collected from shallow and deep oyster cages in the various seasons. Groups encircled in the cluster diagrams represent significantly different communities as identified by PERMANOVA ($p < 0.05$ in all cases).

Table 3.2: Results of an ANOVA considering the effects of the factors included in the GLS model considering Shannon Wiener and Pielou's evenness diversity indices.

Source of variability	df	F-value	p-value
Shannon Wiener diversity index			
Intercept	1	2474.99	p < 0.0001
Season	3	17.39	p < 0.0001
Depth	1	0.43	ns
Orientation	1	0.37	ns
Pielou's eveness index			
Intercept	1	47787.21	p < 0.0001
Season	3	68.20	p < 0.0001
Depth	1	23.10	p < 0.0001

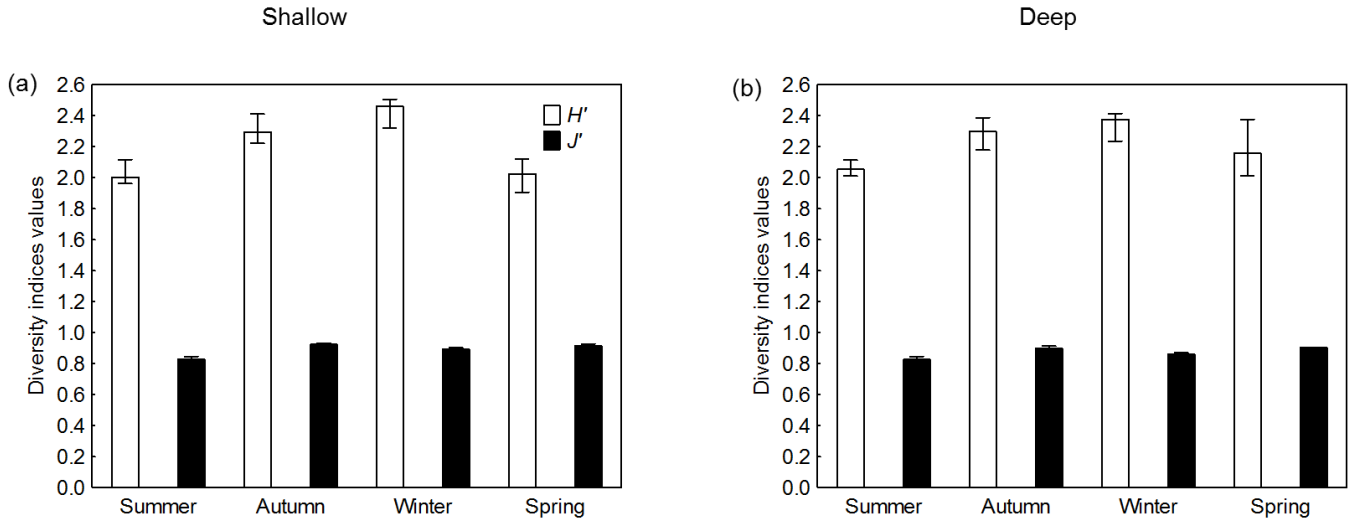

Figure 3.4: Median (± 25%-75% percentiles) Shannon Wiener diversity index (H') and Pielou's evenness index (J') for the biomass (g/m^2) of (a) shallow and (b) deep oyster cages in the four different seasons.

Total density (individuals/m^2) of fouling organisms (as measured by the abundance of unitary organisms) was found to be best explained by season, with no effect of depth or orientation (Table 3.3). Highest densities of fouling organisms were recorded in spring (Table 2, Appendix 3.1). Season and depth were found to best predict total biomass (g/m^2), with both of these factors having a significant effect when considered independently (Table 3.3, Table 2, Appendix 3.1). Significantly lower biomass was recorded on deep cages during autumn than at any other time or depth (Table 2, Appendix 3.1).

Table 3.3: Results of an ANOVA considering the effects of the factors included in the GLS models on total density (individuals/m^2) and biomass (g/m^2) of all fouling organisms.

Source of variability	*df*	F-value	p-value
Total density			
Intercept	1	62.89	$p < 0.0001$
Season	3	38.38	$p < 0.0001$
Total biomass			
Intercept	1	75.77	$p < 0.0001$
Season	3	114.12	$p < 0.0001$
Depth	1	7.95	$p < 0.05$

Both density and biomass of indigenous and alien biota were significantly explained by all three predictor variables. When the effects of these factors on indigenous species were considered independently, only season and depth were found to have significant impacts on both density and biomass (Table 3.4). Density of indigenous

species was lowest on deep cages in autumn (Table 3, Figure 3.5, Appendix 3.1). A similar pattern was recorded for biomass on deep cages, with the lowest biomass recorded during winter (Figure 3.5).

Density of the alien species was explained by season and depth, whilst biomass was explained by depth and orientation (Table 3.4). On the deep cages, the highest densities of these non-native species were recorded in spring, whereas their biomass peaked during summer (Figure 3.5, Table 3, Appendix 3.1). Alien species consistently showed much higher densities and biomass for all four seasons and orientations than did indigenous species (Figure 3.5), although fewer alien species were recorded than indigenous species (Appendix 3.2).

Table 3.4: Results of an ANOVA considering the effects of the factors included in the GLS model on density (individuals/m^2) and biomass (g/m^2) of alien and indigenous species.

Taxonomic groups	Source of variability	df	F-value	p-value
Indigenous				
	Density			
	Intercept	1	26.57	$p < 0.0001$
	Season	3	14.22	$p < 0.0001$
	Depth	1	12.43	$p < 0.05$
	Orientation	1	0.12	ns
	Biomass			
	Intercept	1	39.02	$p < 0.0001$
	Season	3	9.17	$p < 0.0001$
	Depth	1	13.26	$p < 0.05$
	Orientation	1	0.94	ns
Alien				
	Density			
	Intercept	1	60.89	$p < 0.0001$
	Season	3	43.27	$p < 0.0001$
	Depth	1	1.64	ns
	Biomass	1	87.13	$p < 0.0001$
	Intercept	3	116.06	$p < 0.0001$
	Depth	1	9.14	$p < 0.05$
	Orientation	1	0.10	ns

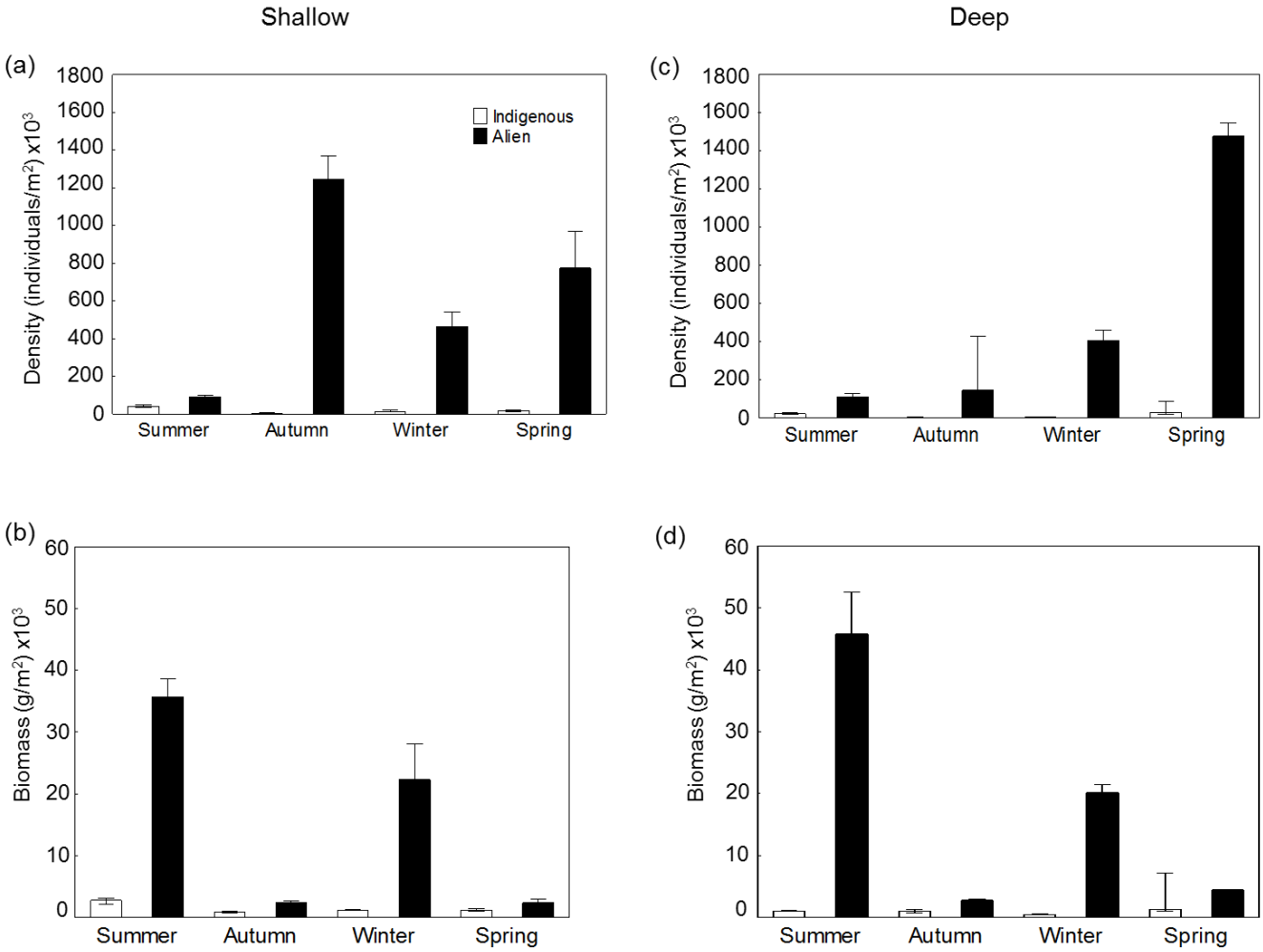

Figure 3.5: Median density (individuals/m^2) (± 25%-75% percentiles) and biomass (g/m^2) (± 25%-75% percentiles) of alien and indigenous species recorded at different depths and seasons.

Of the four alien species that exerted the strongest influence on differences between communities across all seasons, *C. intestinalis* was the only one to occur only in one season (winter; Figure 3.6). Abundance of this species (both density and biomass) was explained by depth and orientation together, but neither of these factors had a significant effect when considered independently (Table 3.5, Table 4, Appendix 3.1). The density and biomass of the amphipod *J. marmorata* was found to be best

explained by season, depth and orientation, with only season having a significant effect when considered independently (Table 3.5, Table 4, Appendix 3.1). Abundance of this amphipod peaked in autumn (Figure 3.6, Table 4, Appendix 3.1). The density (individuals/m^2) and biomass (g/m^2) of the mussel *S. algosus* was found to be explained by all three predictors (Table 3.5, Figure 3.6). Its lowest abundances were observed in autumn in samples removed from the top half of shallow cages (Figure 3.6, Table 4, Appendix 3.1). The density (individuals/m^2) of the mussel *M. galloprovincialis* was also best predicted by season, depth and orientation, whilst biomass was best explained by only season and depth (Table 3.5). The lowest densities of this aggressive invader were recorded on the top half of shallow cages during autumn (Figure 3.6, Table 4, Appendix 3.1).

Table 3.5: Results of an ANOVA considering the effects of the factors included in the GLS model on density (individuals/m^2) and biomass (g/m^2) of the *Jassa marmorata, Semimytilus algosus* and *Mytilus galloprovincialis* samples removed from oyster cages.

Species	Source of variability	*df*	F-value	p-value	Source of variability	*df*	F-value	p-value
Ciona intestinalis								
	Density				*Biomass*			
	Intercept	1	70.81	$p < 0.05$	Intercept	1	6.37	ns
	Depth	1	0.22	ns	Depth	1	0.30	ns
	Orientation	1	1.09	ns	Orientation	1	1.72	ns
Jassa marmorata								
	Density				*Biomass*			
	Intercept	1	26.91	$p < 0.0001$	Intercept	1	23.62	$p < 0.0001$
	Season	3	16.10	$p < 0.0001$	Season	3	15.18	$p < 0.0001$
	Depth	1	3.83	ns	Depth	1	4.74	ns
	Orientation	1	0.73	ns	Orientation	1	0.43	ns
Semmytilus algosus								
	Density				*Biomass*			
	Intercept	1	62.41	$p < 0.0001$	Intercept	1	36.65	$p < 0.0001$
	Season	3	27.12	$p < 0.0001$	Season	3	37.68	$p < 0.0001$
	Depth	1	19.38	$p < 0.0001$	Depth	1	16.19	$p < 0.0001$
	Orientation	1	7.69	$p < 0.05$	Orientation	1	4.05	$p < 0.05$
Mytilus galloprovincialis								
	Density				*Biomass*			
	Intercept	1	72.90	$p < 0.0001$	Intercept	1	26.31	$p < 0.0001$
	Season	3	32.56	$p < 0.0001$	Season	3	93.13	$p < 0.0001$
	Depth	1	11.01	$p < 0.05$	Depth	1	1.74	ns
	Orientation	1	4.30	$p < 0.05$				

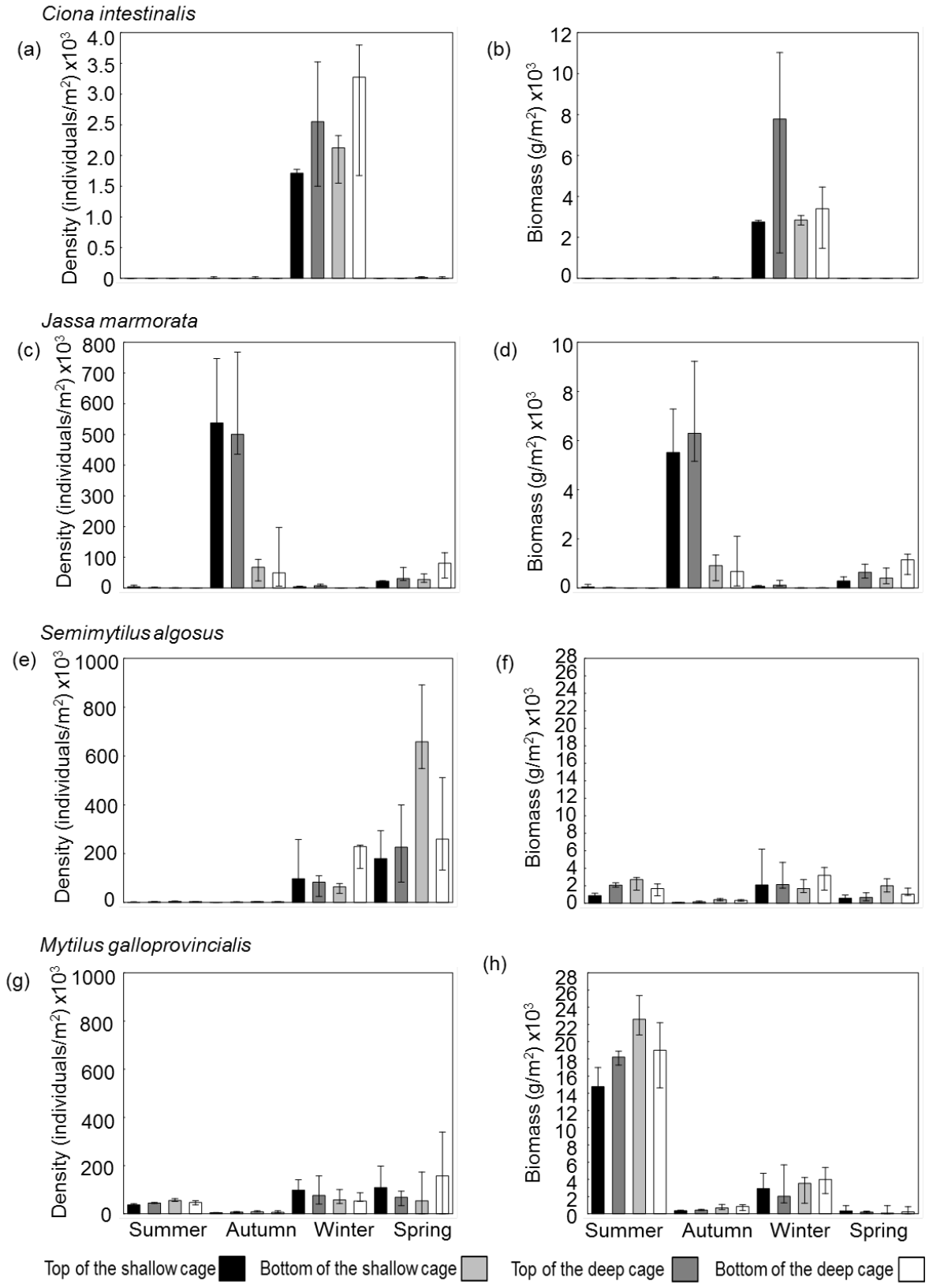

Figure 3.6: Median density (individuals/m^2) (± 25%-75% percentiles) and biomass (g/m^2) (± 25%-75% percentiles) of *C. intestinalis, J. marmorata, S. algosus* and *M. galloprovincialis* recorded at different depths and orientations.

3.4 Discussion

Despite the high shipping volumes (Kruger *et al*., 2005) and the presence of mariculture operations (Weeks *et al*., 1991) within Saldanha Bay and the subsequent potential for the introduction of marine alien species (Bax *et al*., 2003; Coutts *et al*., 2003; Haupt *et al*., 2010), to date no studies have considered temporal changes in the composition of fouling communities in the Bay, or the factors which may drive these changes. It is important that the composition of fouling communities is recorded, to monitor introductions of alien species (Bax *et al*., 2001). This is especially important in Saldanha Bay where harmful alien species may spread to the adjacent West Coast National Park. To better understand fouling communities in Saldanha Bay, this study aimed to document the seasonal changes in the biodiversity of fouling communities associated with oyster cage culture in the Bay.

Results showed that community structure varied significantly between seasons and depth. Such temporal variability in fouling community structure has previously been found to decrease with depth (Ballesteros, 1991; Garrabou *et al*., 2002). Most studies assessing changes in communities with depth have considered benthic communities (as opposed to fouling communities) and generally only focus on a few species (Bak & Luckhurst, 1980; Ballesteros, 1991; Garrabou *et al*., 2002). The

differences in diversity (as reflected by the Shannon-Wiener index) and distribution of individuals among species (as measured by Pielou's evenness index) recorded in this study showed that the diversity of the communities changed between seasons. Studies have found that the settlement and species diversity of fouling communities vary throughout seasons, and that this is due to seasonal changes in temperature (Sutherland, 1974; Mook, 1981; Rico *et al*., 2010; Kripa *et al*., 2012). One of the reasons for temporal changes in fouling communities is the different recruitment periods of the fouling species (Whitlatch, 1977). This seasonal recruitment is thought to be driven by changes in water temperature, which in turn influences the distribution and reproduction of aquatic organisms (Whitlatch, 1977; Kimmerer, 2002). Depth had no significant effect on diversity in this study, although Garrabou *et al.* (2002) found that the Shannon Wiener diversity index increased with depth. This difference may be because the deep cages in this study fell within the shallow spectrum of depths sampled by Garrabou *et al.* (2002).

Depth and season had significant effect on total biomass within fouling communities, with deeper cages supporting significantly more fouling than shallow cages. Reasons for this increase may be that the influences of light and temperature decrease deeper down the water column (Baker & Weber, 1975), thus limiting density of biota (Menge & Sutherland, 1987; Garrabou *et al*., 2002). The low biomass recorded during autumn may be as a result of the low mussel biomass recorded during this time. This is likely a reflection of seasonal variability in mussel recruitment (Griffiths & King, 1979; Gardner & Skibinski, 1990). Although depth did not affect the density of the fouling organisms, this may be because not all individuals included in the total biomass could be recorded individually. The relatively high density recorded during

autumn was as a result of the very high densities of the alien amphipod *Jassa marmorata*. Whereas the relatively high densities recorded during spring were as a result of an increase in settlement of both *J. marmorata* and the indigenous congener *J. slatteryi*, as well as the mussels *Mytilus galloprovincialis* and *Semimytilus algosus*.

Of the 41 species recorded, one was cryptogenic, 31 were indigenous and nine were alien species (Appendix 3.2). Even though there were fewer alien species than indigenous species, they contributed more to both density and biomass when compared to indigenous species. Further studies considering the inter-annual variability of temporal settlement patterns of the recorded alien species would provide a better understanding of when fouling by these could be expected to affect oyster cages most.

The invasive ascidian *Ciona intestinalis* settled predominately during winter, which is characteristic of this species in the southern hemisphere (Scheer, 1945; Millard, 1951). In this study, neither depth nor orientation affected the settlement of this species, which is in contrast to the findings of Chapter 2 and studies by others (Blum *et al*., 2007; Rius *et al*., 2011). These differences could be explained by variability in settlement patterns on different substrata, as this chapter recorded *C. intestinalis* on oyster cages (which provide a three dimensional substrate), while the other studies used flat PVC plates (Blum *et al.*, 2007) and mussel culture ropes (Rius *et al*., 2011).

The amphipod *J. marmorata* inhabited shallow cages at significantly higher densities than deep cages. This is a characteristic of this species (Moura *et al*., 2007), which is often found inhabiting floats and pilings in the waters off British Columbia (Light & Carlton, 2007), where it feeds on phytoplankton and small crustaceans (World Register of Marine Species, 2013). Information on reproductive patterns in this species is not available, but the high abundances recorded in autumn months may reflect the breeding cycle of this amphipod.

To date no sub-tidal settlement studies have been conducted for *S. algosus* along the South African coast. While this study offers a first insight, time constraints precluded consideration of inter-annual variability, but this is an important element that should be considered by future studies. The invasive mussel *M. galloprovincialis* typically has two spawning seasons along the west coast of South Africa, one from March to April and the other from September to October (van Erkom Schurink & Griffiths, 1991). Since clean cages were placed into the water after every sampling session, density and biomass recorded were dependent on mussel recruitment in the period after the clean cages were placed into the water. After a spawning event, large numbers of mussel larvae settle and samples collected at this stage will typically have high numbers but low biomass. In contrast, if settlement occurs soon after the cages are placed in the water, by the time the two month husbandry cycle has been completed few mussels would be present, but these will support greater biomass (Kuenzler, 1961). Subtidal settlement preferences of this species are poorly understood in South Africa and require further investigation.

This study provides some insight into the temporal changes in fouling communities associated with oyster cages in Saldanha Bay. Community composition differed only between season and depth, but not between top and bottom sides of cages. Orientation was also found to have no effect on total density or biomass of fouling biota, however it did have an effect on the two alien mussel species (*S. algosus* and *M. galloprovincialis*). In addition, deep cages supported significantly higher diversity than shallow cages. Although the numbers of alien species recorded was lower than those of indigenous species, alien species were recorded in higher densities and greater biomass. This high abundance of alien species is cause for concern, in light of the close proximity of the oyster farm to the West Coast National Park. These aliens may also impact the culture species of the aquaculture facilities. The high biomass of filter-feeding alien fouling species recorded during summer (*M. galloprovincialis*) and winter (*C. intestinalis*) may have negative impacts on the growth of the cultured oysters. These species not only compete with the oysters for resources, but also foul the mesh cages, reducing water flow through the holes in these cages. It is suggested that monitoring of fouling in the Bay be undertaken so as to track the number of alien species present in the Bay through time.

Chapter 4

The impact of fouling by the alien ascidian *Ciona intestinalis* on growth, condition and survival of farmed Pacific oysters *Crassostrea gigas* in Saldanha Bay

Abstract

Fouling of oyster cages by invasive ascidians has been shown to negatively impact the growth of oysters. *Ciona intestinalis* is a common fouling species affecting the oyster aquaculture operations in Saldanha Bay, South Africa. This experiment aimed to investigate the impact of *C. intestinalis* fouling on growth of cultured oysters and to assess the benefits of four week versus nine week intervals between cage cleaning. The impacts of this ascidian were quantified using shallow (1.5 m) and deep (2.9 m) oyster cages. Temperatures were continuously recorded for shallow and deep cages for the full nine weeks. After four weeks oysters were taken out of the cages of the complete removal group and placed into clean cages, *C. intestinalis* was removed from the *Ciona* removal cages and emersion control cages were removed from the water for the time it took to remove the *C. intestinalis* individuals from the *Ciona* removal cages. Control cages were left undisturbed for the full nine weeks. *C. intestinalis* had no impact at low abundances. Temperature had no measurable impact on oyster growth. Oysters in shallow cages gained on average 32% (control), 29% (emersion control), 25% (*Ciona* removal) and 30% (complete removal), whilst oysters in the deep cages gained 38% (control), 39% (emersion control), 35% (*Ciona* removal) and 32% (complete removal) on average. Cleaning cages after four weeks instead of nine weeks is detrimental to oyster growth. Future studies are required to determine impacts of *C. intestinalis* at high abundances.

4.1 Introduction

Aquaculture is an internationally important industry which plays a critical role in supplying sources of protein to low income countries (Fitridge *et al*., 2012). Marine aquaculture (mariculture) focuses predominately on shellfish and finfish culture. Bivalve culture provides job creation opportunities, as well as food security through the mitigation of poverty (Olivier *et al*., 2013). The most commonly cultured shellfish organisms are oysters, mussels and scallops (Fitridge *et al*., 2012). The infrastructure used in shellfish culture includes equipment such as cages, ropes, weights, floats and high density polyethylene cages. Since this infrastructure is exposed to the marine environment for long periods of time, it is susceptible to fouling by epibionts like sessile filter-feeding ascidians and mussels, and infauna like polychaetes and amphipods. These fouling organisms are problematic for oyster farmers, as filter-feeding species compete with cultured oysters for oxygen and nutrients (Lesser *et al*., 1992; Lodeiros & Garcia, 2004), and reduce water flow through the cages (Pit & Southgate, 2003; Denny, 2008; Kripa *et al*., 2012; Cordell *et al*., 2013). This competition negatively impacts efficient production (Rikard *et al*., 1996; Greene & Grizzle, 2007), and can cause severe direct economic losses (Adams *et al*., 2011). Expenses related to biofouling removal account for 5-15% of total production costs in shellfish culture (Adams *et al*., 2011). Several strategies to control fouling on cages have been investigated, including pressure-washing of cages (Dafforn *et al*., 2011; Arens *et al*., 2011), the use of antifouling substances (e.g. copper, chlorine and acetic acid) (Watson *et al.,* 2009), introductions of natural predators of the fouling species, such as crabs and sea urchins (Rikard *et al*., 1996; Lodeiros & Garcia, 2004, Braithwaite *et al*., 2007).

Oyster culture cages are designed to allow for maximum water flow to optimize oyster production. However once fouled, water flow is reduced (Claereboudt *et al*., 1994) and in extreme cases the weight of fouling organisms can physically damage the cages by increasing drag. Where swells or tides are strong, heavily fouled cages and the stock within them may be lost (Swift *et al*., 2006). Fouling species may also settle on the oyster shells (Watson *et al.,* 2009), the fouling of oyster shells can negatively impact growth of the bivalve (Carver *et al*., 2003; Daigle & Herbinger, 2009) and can cause shell deformities (Taylor *et al*., 1997). Fouling increases the occurrence of diseases by reducing the water flow through cages, resulting in a build-up of faecal matter and the lower levels of oxygen present provide an ideal habitat for bacterial growth (Costelloe *et al*., 1996). The main food source of filter-feeding species, such as oysters and ascidians, is phytoplankton and the abundance of phytoplankton can be measured as the concentration of chlorophyll *α* in the water column (Gangnery *et al*., 2003). A decrease in planktonic food within the cages is also coupled with the decrease in water flow, with limited food availability causing poor growth and increased mortalities within cultured bivalve species (Uribe & Etchepare, 2002). Numbers of biotoxin-producing phytoplankton species may also increase around fouled structures, (Ross *et al*., 2002), influencing the health of cultured bivalves and making them unfit for human consumption.

The nutrient-rich Benguela Upwelling system provides a favourable environment for filter-feeding Pacific oysters (*Crassostrea gigas*) and Mediterranean mussels (*Mytilus galloprovincialis*) both of which are cultured in Saldanha Bay (Heasman *et al*., 1998). The food availability in this system also provides favourable conditions for the settlement of other filter-feeders, such as ascidians (Pitcher & Calder, 1998). The

high shipping volume in Saldanha Bay (Kruger *et al*., 2005) can result in the introduction of alien fouling species, which will then foul the oyster cages within the Bay (Masson *et al*., 2013). Ascidians are one of the most prominent fouling species on shellfish cages worldwide (Rocha *et al*., 2009). One of the most common species on aquaculture gear is the vase tunicate *Ciona intestinalis* (Hecht & Heasman, 1999; Ramsay *et al.*, 2009; Rius *et al*., 2011). The larvae of this species have a strong preference for settling on mariculture structures, possibly due to the low light and water movement associated with cages (Schmidt & Warner 1984). *C. intestinalis* fouling, on culture cages of filter feeding species, has profound negative impacts on the growth of cultured organisms (Carver *et al*., 2003; Daigle & Herbinger, 2009). *C. intestinalis* decreases the overall size and increases mortality rates of cultured mussels (Daigle & Herbinger, 2009). Due to the fouling of mussel sleeves by *C. intestinalis*, mussel farms in Saldanha Bay re-sleeve their mussel stocks after the seasonal settlement of this ascidian (Hecht & Heasman, 1999). Fouling by this species impacts mussels farming in Saldanha Bay, as it reduces growth of the target mussels (Robinson *et al*., 2005a; Rius *et al*., 2011). Because of its high fecundity and reproductive rate, *C. intestinalis* recruits onto oyster stacks (see Chapter 3) in such high densities that it can reduce water flow through cages (Gray & Christie, 1983). In other countries this species is physically removed by scraping, high pressure water spraying, or scrubbing by hand (Arakawa, 1990; Clancey & Hinton, 2003).

This chapter aimed firstly to document the impacts of *C. intestinalis* fouling of oyster cages on oyster growth in Saldanha Bay and secondly to assess the benefits of four week versus nine week intervals between cage cleaning.

4.2 Methods

Study site

This study took place on the oyster lines of the West Coast Seaweeds, these lines are situated in Small Bay (Figure 4.1).

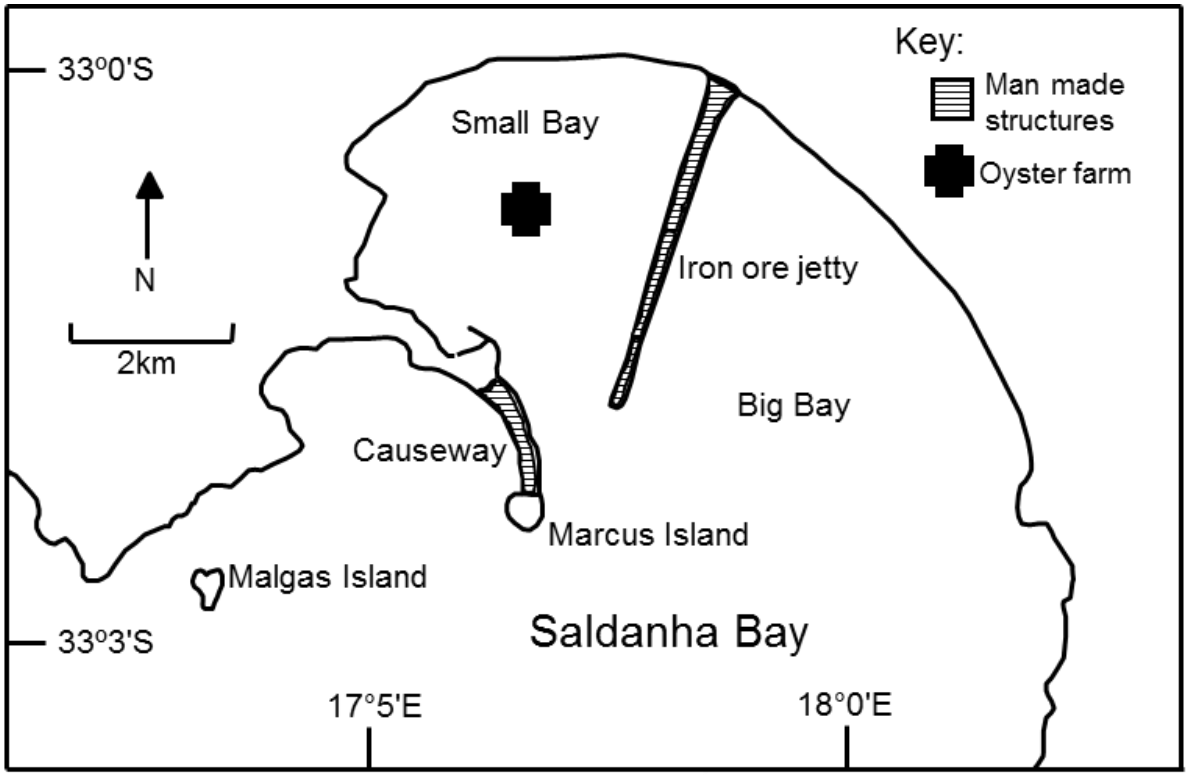

Figure 4.1: Map of Saldanha Bay indicating the position of West Coast Seaweeds oyster lines in Small Bay.

Experimental design

The impact of fouling by *Ciona intestinalis* was assessed over a period of nine weeks, from June to August 2013. This time period replicates the husbandry cycle of West Coast Seaweeds. Four treatments groups were considered, each on a separate oyster stack. The first (complete removal) involved the emersion of the oyster cages and the transfer of oysters, after four weeks, into clean cages. The second treatment (*Ciona* removal) involved the emersion of the oyster cages and the removal of all *C. intestinalis* from cages, after four weeks of being placed into the water. The combination of these treatments assisted in the analysis of the impacts of *C. intestinalis*. Control oysters in the third stack were exposed to no cleaning and no emersion for nine weeks and the emersion control oysters in the fourth stack were removed for the same length of time that it took to remove the *C. intestinalis* and other fouling species from treatment groups. The *C. intestinalis* removed from both treatment groups were counted and weighed to the nearest milligram. Removal of most fouling species and *C. intestinalis* were done by hand, whilst barnacles were chiselled off. The experiment was replicated using the shallow (approximately 1.5 m from the water surface) and deep cages (approximately 2.9 m from water surface) of four stacks.

A set of 800 oysters were weighed to the nearest milligram and marked using numbered tags and epoxy glue. The individually-marked oysters were then weighed and 100 were placed into each of the shallow and deep cages (Figure 4.2). There were four stacks in total (one for each treatment group), each with a shallow and a deep cage (Figure 4.2). Five kilograms of oysters (each oyster weighed

approximately 40-49 g) were placed in each of the three cages between the top and bottom cages, to weight the stacks and replicate culture conditions. After nine weeks, all oysters were harvested and re-weighed to assess percentage weight gain.

The percentage weight gained in grams (% Weight gained (g)) was calculated as:

$$\%\text{Weight gained (g)} = \left(\frac{\text{End weight (g)} - \text{Starting weight (g)}}{\text{Starting weight (g)}} \right) * 100$$

Forty oysters from each of the eight cages were used for representative assessment of Dry Weight Condition Index (DWCI) and shell density.

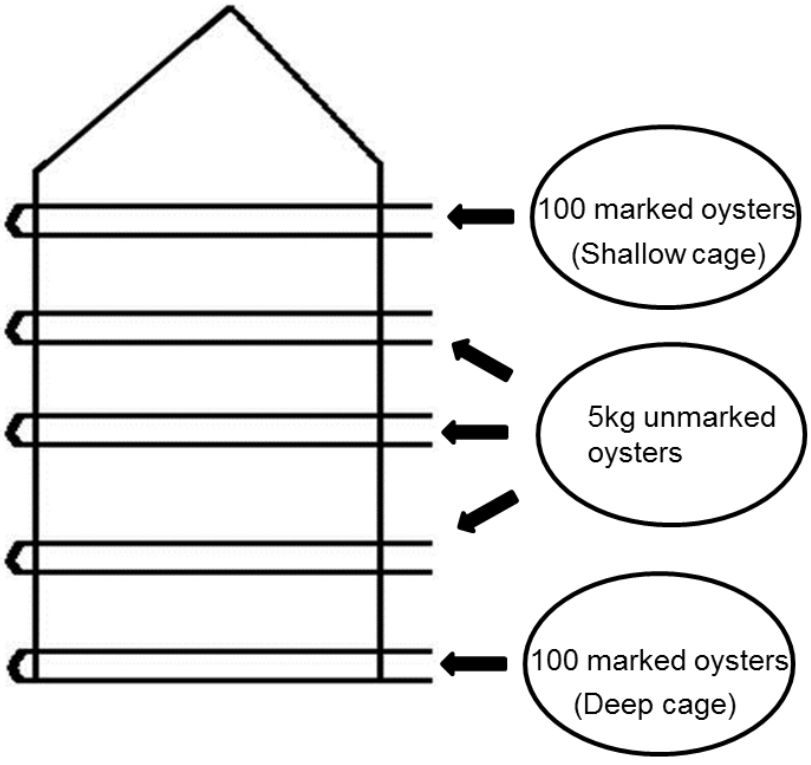

Figure 4.2: Oyster stack and the placement of the oysters in the shallow and deep cages in order to assess the impact of *Ciona intestinalis* fouling on oyster growth and condition.

Growth was measured at the end of the two month experiment and expressed as weight gain (g) per 100 oysters. For DWCI, oysters were weighed whole, shucked, and their meat and shells weighed separately, wet. After drying at 50°C for five days, the dried shells and meat were re-weighed. This information was used to calculate DWCI and shell density, which were chosen because of both their wide use in the Pacific oyster literature, and their independence from variability in inter-valval fluid volume (Pogoda *et al*., 2011). DCWI was calculated using the following equation (Handley, 2002):

$$DWCI = \left(\frac{\text{Meat dry weight}}{\text{Shell dry weight}} \right) * 1000$$

Shell density was measured using the equation (Robert *et al*., 1993):

$$\text{Shell density} = \left(\frac{\text{Shell dry weight}}{\text{Shell wet weight}} \right) * 100$$

Sea temperature in the shallow and deep cages was recorded every 30 min during the experiment, using Thermochron iButton recorders in waterproof plastic bottles attached to the inside of the cages. iButton loggers were verified at 0°C and 21.5°C against two instruments: a MajorTech MT605 digital thermometer (Isando, South Africa) and a liquid-in-glass thermometer, which showed agreement to within 0.05°C

over this range. Values obtained from two loggers that showed deviations of 0.25°C and 0.125°C, were adjusted accordingly.

Statistical analysis

Univariate measures (i.e. percentage weight gained by each oyster during the experiment, DWCI, shell density, and inter-cage temperature differences) were compared between the control, emersion control, *Ciona* removal and complete removal treatment groups and two depths using either two-way ANOVAs or Kruskal-Wallis ANOVAs, depending on the results of the Shapiro-Wilks normality test.

DWCI was compared between each treatment group, and between depths within treatment group. Ratio-based analysis was used because oysters were selected to be of similar size and weight and therefore had a narrow range of differences, reducing the power of, and removing the need for, regression-based comparisons, such as analyses of covariance.

4.3 Results

No *C. intestinalis* individuals were present on the *Ciona* removal treatment cages after four weeks of being placed back into the water. After nine weeks, only 11 individuals were found on the deep cage of the *Ciona* removal and eight individuals on the deep cage of the emersion control cage, there were no *C. intestinalis* present on any of the other cages. As expected from previous studies (Chapter 3; J. Jonker,

unpublished data) deep cages supported more fouling biomass than shallow cages, with mean biomass of 20.5 g/m^2 and 14.1 g/m^2 respectively.

Shallow cages experienced significantly higher sea temperatures during the experiment, than deep cages (H_1 = 196.7, N = 24192, p < 0.05; Figure 4.3). The mean difference between the median temperatures of shallow and deep cages was 0.09^{o}C, with a maximum difference of 0.13^{o}C. The mean difference between median temperatures of the four treatment groups for shallow cages was 0.22 ^{o}C with a maximum difference of 1.48 ^{o}C and for deep cages 0.24 ^{o}C, with a maximum difference of 1.45 ^{o}C. There were no significant differences between temperatures of the control and emersion control of the shallow cages (Kruskal-Wallis ANOVA, H = 111.1, p > 0.05; Figure 4.4a). However, the control had significantly higher temperatures than *Ciona* removal and complete removal (in both cases p < 0.05). The complete removal had the lowest temperatures of all treatments (in all cases p < 0.05). Within deep cages, all four treatment groups differed significantly, with the highest temperatures being experienced by the treatment emersion control and the lowest in the complete removal (H_3 = 161.6, N = 12096, p < 0.05; Figure 4.4b).

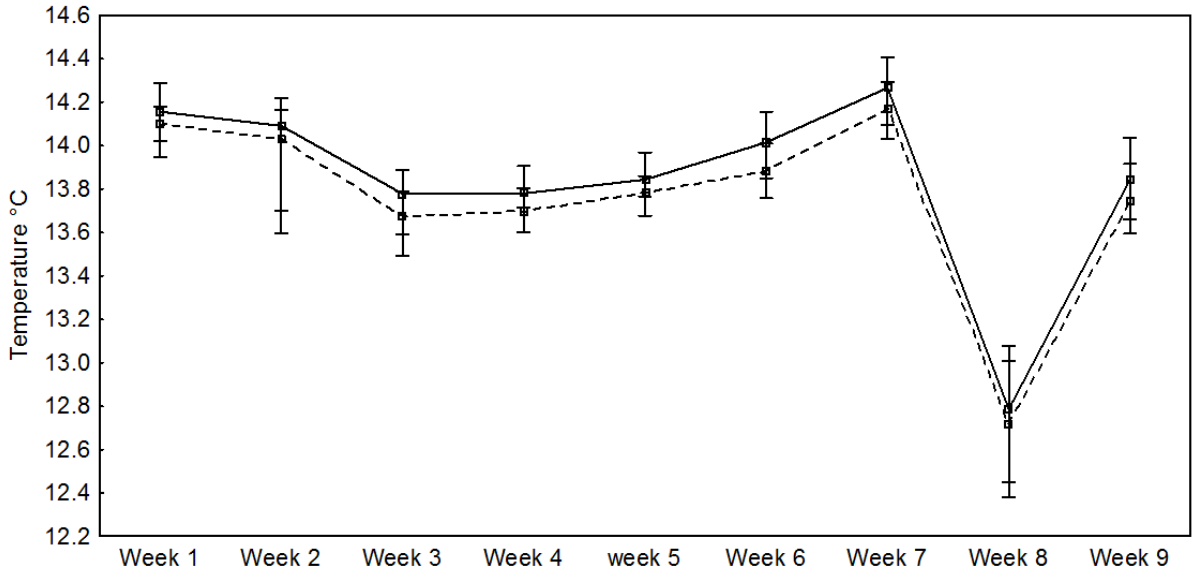

Figure 4.3: Weekly median, quartiles (25%-75%) and ranges of sea temperatures (°C) for shallow and deep cages, during this experiment. Shallow cages are indicated by the solid line and deep cages by the dashed line. The temperature spike observed during week eight, may be the result of wind driven mixing of warmer surface water and cold deep water after an upwelling event.

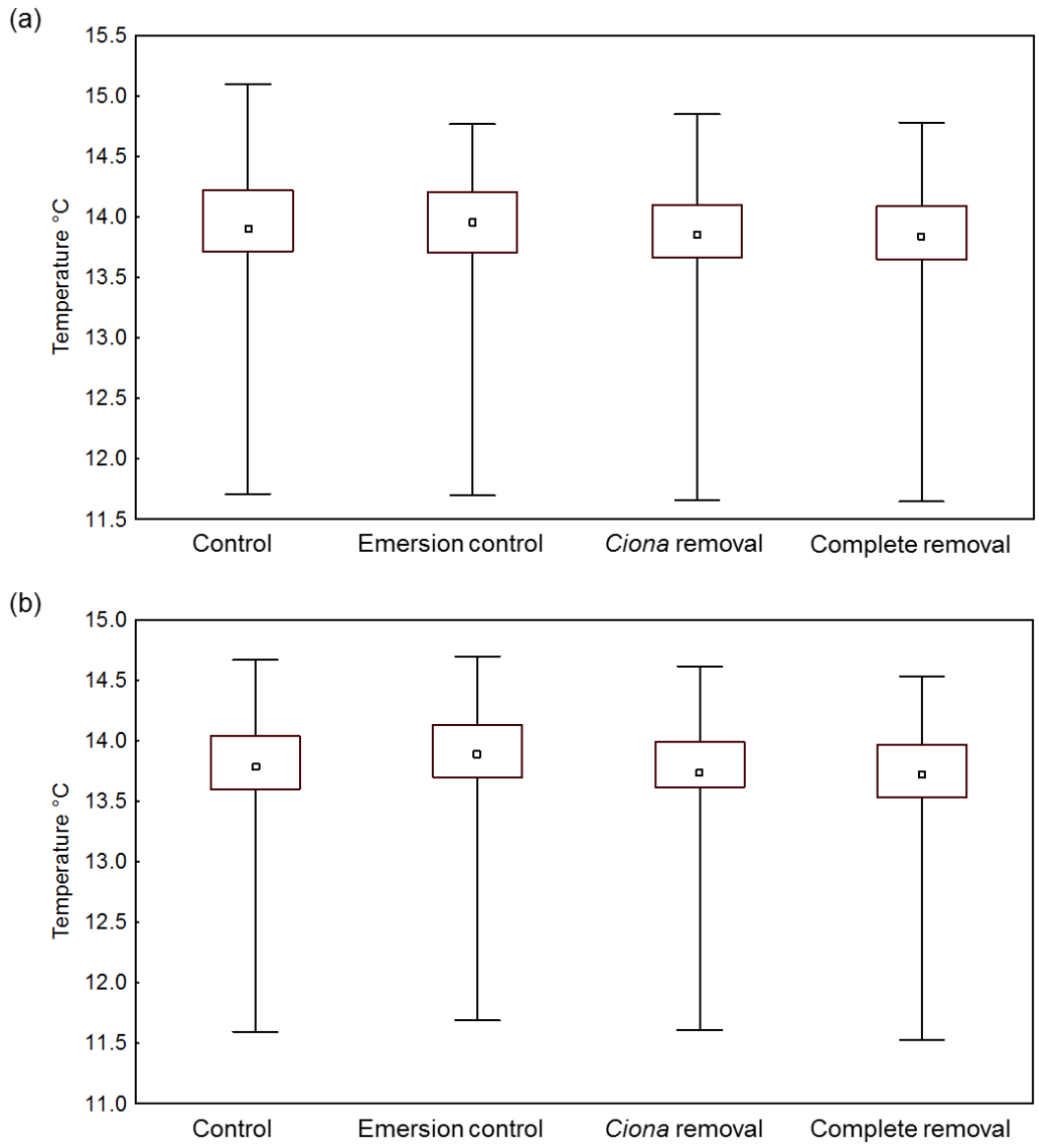

Figure 4.4: Weekly median, quartiles (25%-75%) and ranges of sea temperatures (°C) of the four treatments for (a) shallow cages and (b) deep cages, recorded over nine weeks.

The effects of depth and treatment on the percentage weight gain of oysters and the interaction between these factors were all significant (in all cases $p < 0.05$; Table 4.1). Oysters in deeper cages gained more weight than those in the shallow cages (Figure 4.5). After nine weeks, oysters in shallow cages had gained on average 32%

(control), 29% (emersion control), 25% (*Ciona* removal) and 30% (complete removal). Similarly for the deep cages, oysters gained on average 38% (control), 39% (emersion control), 35% (*Ciona* removal) and 32% (complete removal). Comparisons between the four treatments of the shallow cages showed that control oysters gained significantly more weight than oysters of the *Ciona* removal group (Tukey test, $p < 0.05$). Within deep cages, oysters of the emersion control gained significantly more weight than the complete removal (Tukey test, $p < 0.05$).

Table 4.1: Results of factorial ANOVA on the effects of depth and treatment on percentage weight gained (g) by oysters.

Source	df	MS	F	*p*
Depth	1	10253.4	54.6	$p < 0.0001$
Treatment	3	1022.6	5.4	$p < 0.05$
Depth*Treatment	3	736.7	3.9	$p < 0.05$

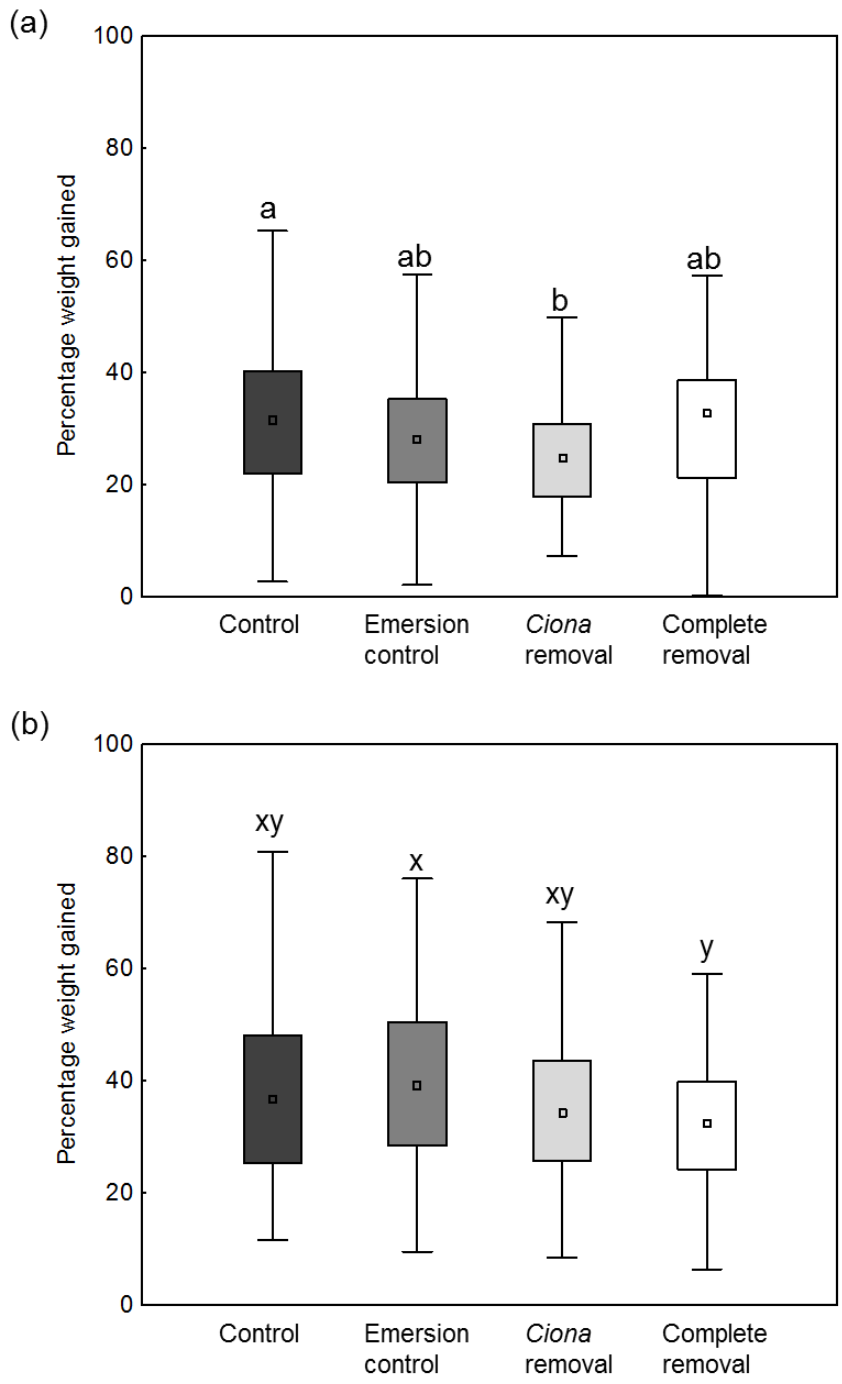

Figure 4.5: Median, quartiles and ranges of percentage weight gained for (a) shallow and (b) deep cages for the four treatments. Letters which differ above the box and whisker plots indicate significant differences between treatments ($p < 0.05$).

Within the shallow cages, significant differences were recorded between DWCI among the four treatment (Kruskal-Wallis ANOVA, H_3 = 29.4, N = 160, $p < 0.05$). Oysters subjected to the emersion control treatment had significantly greater DWCI than the control and complete removal groups ($p < 0.05$, Figure 4.6a). Complete removal groups did not differ significantly from control groups ($p > 0.05$). In contrast, there were no significant differences between DCWI for the four treatments in deep cages (Kruskal-Wallis ANOVA, H_3 = 2.6, N = 160, $p > 0.05$) (Figure 4.6b).

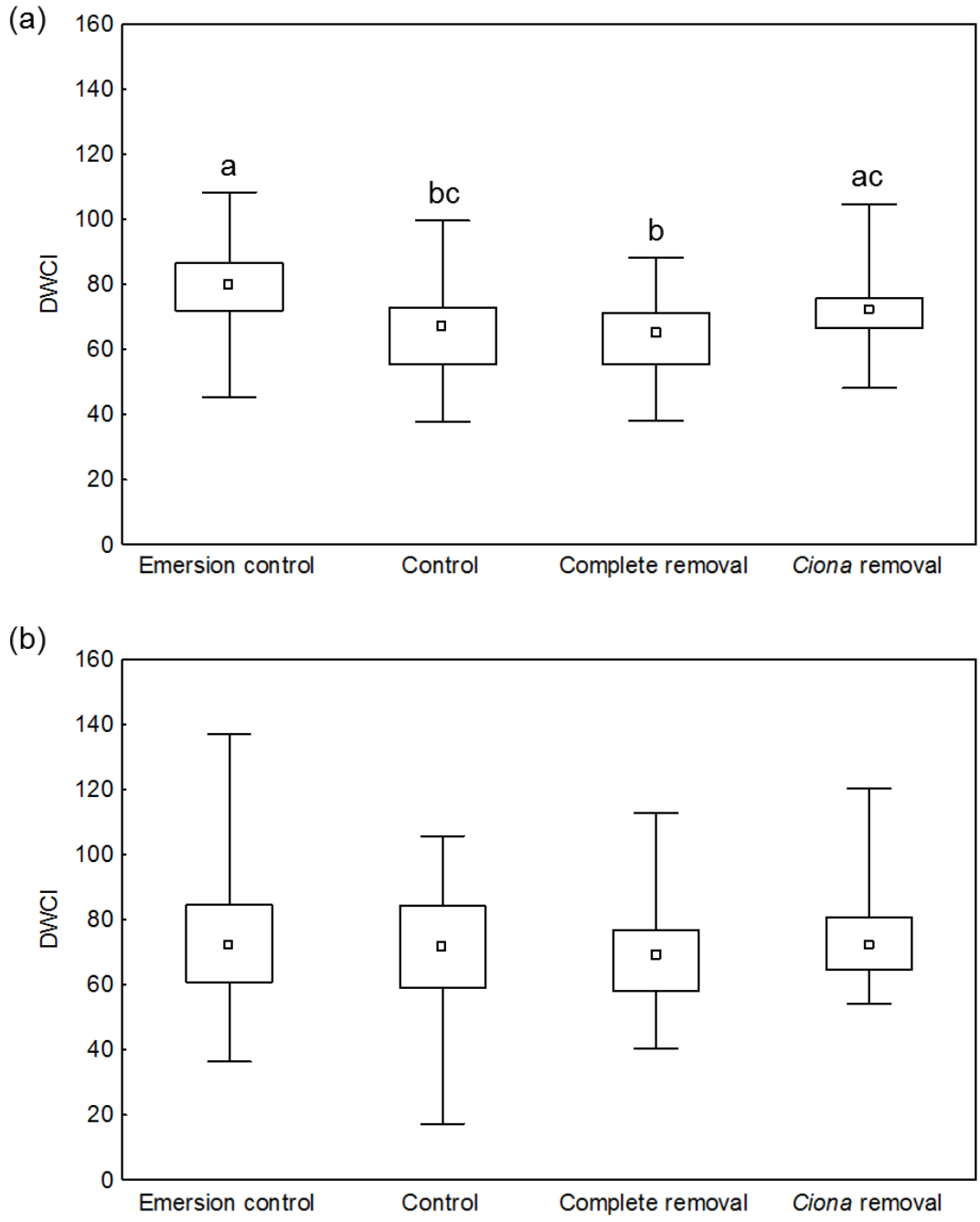

Figure 4.6: Median, quartiles and ranges of DWCI for (a) shallow cages and (b) deep cages for the four treatments. Letters which differ above the box and whisker plots indicate significant differences between treatments ($p < 0.05$).

Significant differences in shell density were observed among the treatments for shallow cages (Kruskal-Wallis ANOVA, $H_3 = 23.9$, N = 160, $p < 0.05$). The oysters in the control had significantly higher shell density than those oysters in the emersion control and complete removal treatments ($p < 0.05$) (Figure 4.7a). There were no significant differences between shell density of the four treatments for deep cages (Kruskal-Wallis ANOVA, $H_3 = 5.2$, N = 160, $p > 0.05$, Figure 4.7b).

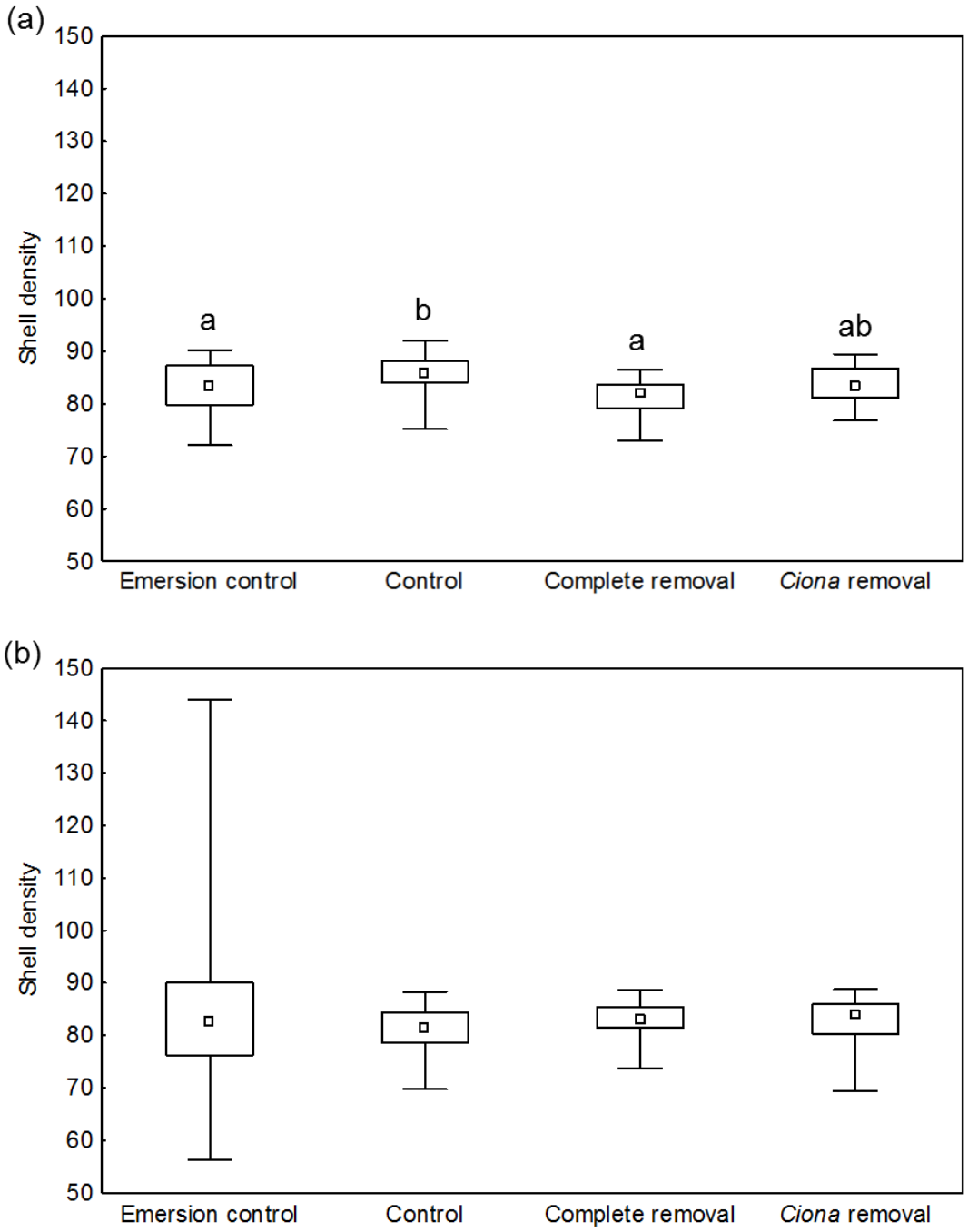

Figure 4.7: Median, quartiles and ranges of shell density for (a) shallow cages and (b) deep cages for the four treatments. Letters which differ above the box and whisker plots indicate significant differences between treatments ($p < 0.05$).

4.4 Discussion

Ciona intestinalis is a common fouling species on the mussel ropes in Saldanha Bay (Hecht & Heasman, 1999; Rius *et al*., 2011) and has negative impacts on oyster growth in North America (Uribe & Etchepare, 2002; Carver *et al*., 2003; Daigle & Herbinger, 2009). To date, however, there have been no studies considering the impact of this species on the oysters farmed in Saldanha Bay. As South African oyster culture is focused in Saldanha Bay (Olivier *et al*., 2013), impacts of fouling by *C. intestinalis* on the growth of oysters is of interest. As labour is one of the largest expenses incurred by culture facilities (Carver *et al*., 2006) and cleaning cages is labour-intensive (Hodson *et al*., 1997), it is advantageous to know the benefits of only *C. intestinalis* and/or complete fouling removal on oyster growth. This study shows that it is more beneficial to keep the oyster cages in the water for the full two months, rather than to clean them after four weeks.

During this study *C. intestinalis* abundance was lower than in other winters in the past (A.F.G. Tonin *pers. comm*.). These individuals were found on only two of the four stacks used in the experiment. In contrast, an experiment that sampled fouling species from the same oyster cages situated in Big Bay, during January, April, July and October of 2012 yielded a mean density of 2246 individuals/m^2 and a mean biomass of 4188. g/m^2 between the six stacks sampled (Chapter 3). This suggests high inter-annual variability of *C. intestinalis* abundance in Saldanha Bay, also observed by Rius *et al.* (2011). This variability may also be linked to the variable trophic conditions typical of the West coast. Similarly to Chapter 2, the *C. intestinalis* individuals recorded on the oyster cages were on the underside of the deep cages.

Though the temperatures recorded for the shallow cages were significantly higher than those recorded in deep cages, the temperature difference between levels was 0.1 °C, with mean temperatures for shallow and deep cages of 13.8 and 13.7°C respectively over the entire study period). The optimal sea temperature range, which promotes high growth in *Crassostrea gigas* culture, is from 11°C to 19°C (Walne, 1979; Mann *et al*., 1991; Bougrier *et al*., 1995; Shatkin *et al*., 1997). The small temperature differences were unlikely to be large enough to influence percentage weight gained over such a short period. The sharp drop in temperature observed for both shallow and deep cages during week eight, may reflect the effects of an upwelling event in this area. This event occurs from August to May over the coastal shelf at the mouth of Saldanha Bay and involves the wind-driven upwelling of nutrient rich cold water (Monteiro & Largier, 1999). This upwelled water is then pushed into the Bay and results in an increase in available nutrients, which may cause an increase in biofouling on oyster cages, as well as an increase in oyster growth.

Due to no *C. intestinalis* individuals being present on the cages after one month in the water, there were no differences in handling techniques between the *Ciona* removal and emersion control groups. Therefore, no significant differences in percentage weight gained were observed between these groups, for both shallow and deep cages. Overall, oysters in the deep cages gained more weight than those in shallow cages, which agrees with results found by Pieterse *et al*. (2012). Among the shallow cages, the control gained the highest percentage weight. Growth of

oysters in shallow cages is usually erratic, which could be attributed to these oysters being in closer proximity to the water surface, thus exposed to fluctuating abiotic conditions when compared to the oysters in deep cages. Regarding the deep cages, the oysters in the complete removal treatment gained the least percentage weight, which was not significant when compared to the control. This may be attributed to the physical stress placed on the oysters whilst they are removed from the cages by vigorously shaking the cages to dislodge the oysters and the oysters dropping into crates.

For shallow cages, emersion control oysters had the highest DWCI values and the control and complete removal groups had the lowest. The oysters in the emersion control of the shallow cage also had low shell density values. Since DWCI is a ratio between meat and shell mass, this indicates that emersion control had higher meat mass than shell mass. The oysters in the complete removal group of the shallow cage had the lowest DWCI and the lowest shell density. This may indicate that the abrasive stress of being physically dislodged from the cages, negatively impacted the oyster's meat mass. This decrease is because during stressful periods, immune response messengers such as hormones and corticosteroids divert energy away from non-essential functions like reproduction and growth (Chrousos & Gold, 1992; Ottaviani & Franceschi, 1996). As little as 15 minutes of mechanical shaking of 60-70 g Pacific oysters has been shown to up-regulate the numbers and activity of circulating haemocytes, and increase reactive oxygen species production in the two hours following application of the stress (Lacoste *et al*., 2002).

The shell density values of the oysters in the shallow cages were highest in the control group. Since these oysters were left untouched in the water for the duration of the experiment, it appears that removing oysters from the water decreases shell density. Previous studies found that an increase in stress (e.g. high temperatures and/or physical abrasive stress) resulted in increased shell density (Walne & Mann, 1975; Brown & Hartwick, 1988; Shpigel & Blaylock, 1991). Stressors such as mechanical shaking of oyster cages can negatively affect the growth rates of cultured oysters (Lacoste *et al*., 2002). It was expected that the shell density would be higher in oysters of the complete removal groups, because oysters use shells to protect themselves against predators and the mechanical shaking would damage the shells.

The results from this study provide important information for managers of oyster farms in Saldanha Bay. During this study the fouling biomass was relatively low and was predominately recorded on deep cages. Also, *C. intestinalis* settlement during the course of the experiment was so low that impact analysis could not be conducted. Oysters in deep (2.9 m) cages gained significantly more weight than those in the shallow (1.5 m) cages. J. Jonker found that oysters gained the most weight at a depth of five meters and therefore it would be beneficial for farmers to setup cages from 2.9 m to 5 m, in order to cover both of these depth ranges. It would be useful to conduct further growth studies at multiple depths, seasons and years. Future studies should assess the inter-annual variability for the settlement of *C. intestinalis* in Saldanha Bay.

At low densities *C. intestinalis* had no impact on oyster growth. From the results of this study, it can be suggested that during years of low fouling biomass, the removal of oyster cages after one month of being placed into the water has a negative effect on weight gain by oysters. Therefore it would be more beneficial if the oyster cages remained in the water for the full two months. Once settlement cycles of *C. intestinalis* in Saldanha Bay are better understood, this study should be replicated in order to document any potential impact by fouling of this species on the cultured oysters.

Chapter 5

General Conclusion

Invasive alien species are predominantly introduced into new environments, by three vectors: ship hull fouling (Griffiths *et al*., 2009); ballast water (Bax *et al*., 2003; Mead *et al*., 2011a); and mariculture (Galil, 2007). These introduced species can have profound negative impacts on the ecology and economy of their receiving environment (Grosholz *et al*., 2000; Vila *et al*., 2010). Despite more than 80 introduced species being known from South Africa, only 5% of these have been considered for their potential impact (Mead *et al*., 2011a). This is most concerning in the Saldanha Bay system, which is ecologically important as it hosts the West Coast National Park (Weeks *et al*., 1991). Saldanha Bay could be particularly vulnerable to introductions due to the high shipping volume experienced and several aquaculture and fish manufacturing facilities situated within it (Kruger *et al.*, 2005). Of the numerous fouling species found within Saldanha Bay, *Ciona intestinalis* is considered to be one of the most common species impacting aquaculture infrastructure (Hecht & Heasman, 1999; Ramsay *et al.*, 2009; Rius *et al*., 2011).

C. intestinalis did not settle on plates placed in an area with high water movement, however they did settle on plates in an area with low water movement. This is a clear indication that water movement does moderate the settlement of this species, which agrees with previous settlement studies (Howes *et al*., Mead *et al*., 2011a). The settlement of *C. intestinalis* was also unexpectedly low during the study presented in Chapter 2 and varied in abundance between pates. This may be due to a decrease in the abundance of this species in Saldanha Bay, or inter-annual variability in

spawning times as observed in other countries (Keough, 1983). This species also settled predominately on the underside of the deep plates placed in areas with low water movement. These settlement patterns made statistical comparisons between shallow and deep plates impossible. It was anticipated from previous impact studies (Blum *et al*., 2007) that this species would negatively affect species richness. However, results from Chapter 2 found that *C. intestinalis* does not significantly affect the structure or diversity of fouling communities. This may be due to the very low abundance of *C. intestinalis* recorded during this work.

Results from Chapter 3 showed that the community structure of the fouling communities present on oyster cages differed significantly between the four seasons and the two depths, but did not differ between orientations. The diversity of these fouling communities also differed significantly between seasons, with deeper cages having higher species diversity than shallow cages. The overall fouling biomass (g/m^2) on the cages was most significantly impacted by both season and depth, but not orientation. Orientation only influenced the settlement of the two alien mussels *Mytilus galloprovincialis* and *Semimytilus algosus*. The deep cages supported significantly more fouling biomass than shallow cages. Fouling biomass was highest for summer (due to a presence of relatively large mussels both *M. galloprovincialis* and *S. algosus*) and lowest during autumn (due to a low abundance of these mussels).

C. intestinalis settlement was low during the course of the experiment conducted in Chapter 4. At low abundance, this species was shown to have no impact on the

growth of cultured oysters. Depth had a significant effect on oyster growth and fouling, with oysters in the deep cages gaining significantly more weight than those in the shallow cages and deep cages supporting higher abundances of fouling. J. Jonker also found that the fouling on cages and weight gained by oysters increased deeper down the water column until a depth of about 5 m. No explanation can be provided for this result, however, future growth studies considering factors such as influence of abiotic conditions and concentrations of phytoplankton may fill the gap in our knowledge. Shallow cages experienced significantly higher temperatures than deeper cages, although the difference was not large and fell within the range considered ideal for optimum growth of *Crassostrea gigas*. The results from this study also showed that during seasons of low abundance of fouling, regular cleaning of oyster cages is detrimental, rather than beneficial.

In conclusion, the results found within this study show that at low abundances, *C. intestinalis* has no impact on either the structure of indigenous fouling communities, or the growth of cultured oysters. Important to note from both Chapter 2 and Chapter 3 was that, although there were more indigenous species than alien, the alien species contributed significantly more to the overall biomass recorded during these two studies. Furthermore, the abundances of *C. intestinalis* recorded during 2012 (Chapter 2 & 3) were lower than those recorded during 2013 (Chapter 4). This difference may indicate inter-annual variability with regards to settlement of this invasive fouling species. This shows that longer-term studies and monitoring are required.

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Appendix 2.1: A list of biomass (g/plate ± 1 SE) of fouling species present on perspex plates after completion of experiment. Aln= Alien species, Ind= Indigenous species, Unid= Unidentified species and Cryp= Cryptogenic species.

Taxonomy	**Status**	**Control** Mean biomass±(SE)	**Treatment Control** Mean biomass±(SE)	**Treatment** Mean biomass±(SE)
Amphipoda				
Lysianassa ceratina	Ind	0.00496±0.0038	0.07458±0.06054	0.000417±0.000417
Jassa marmorata	Aln	0.03108±0.00984	0.03254±0.00865	0.055375±0.019499
Caprella equlibria	Cryp	0.14771±0.07295	0.13542±0.04329	0.091667±0.042861
Phtisica marina	Ind	0.00017±0.00013	0.00071±0.00044	0.000125±0.000125
Paramoera capensis	Ind	0.00988±0.00334	0.08625±0.04216	0.009500±0.003694
Cymadusa filosa	Cryp	0.00042±0.00042	0.00083±0.00083	0.003750±0.002998
Tanaidacea				
Anatanais gracilis	Ind	0.00500±0.00168	0.00867±0.00386	0.001167±0.000567
Isopoda				
Paridotea reticulata	Ind	0.18167±0.15292	0.91146±0.75345	0.006083±0.002505
Mysidacea				
Unidentified shrimp	Unid	0.00013±0.00007	0.00008±0.00008	
Decapoda				
Hymenosoma orbiculare	Ind	0.00363±0.00153	0.02417±0.01016	0.005083±0.002818
Crayfish	Ind			0.012917±0.012917
Bivalvia				
Mytilus galloprovincialis	Aln	0.02463±0.01432	0.08125±0.05188	0.017833±0.010427
Semimytilus algosus	Aln	0.05833±0.03755	0.00375±0.00375	0.000417±0.000417
Venerupis corrugatus	Ind	0.00125±0.00125		
Crassostrea gigas	Aln	0.01692±0.00794	0.01158±0.0052	0.006667±0.003791

Opisthobranchia				
Janolus capensis	Ind	0.05708±0.02797	0.05825±0.03366	0.038458±0.02959
Gastropoda				
Crepidula porcellana	Ind		0.00125±0.00125	
Ascidiacea				
Ciona intestinalis	Aln	50.75333±47.5728	57.18208±52.7518	2.854833±1.600945
Botryllus schlosseri	Aln	2.2625±1.46368	3.99292±2.36381	0.552917±0.409563
Clavelina lepadiformis	Aln	0.005±0.005	0.79583±0.79583	0.065±0.062025
Botryllus magnicoecus	Ind	2.015±0.96642	3.01963±1.49068	2.18875±1.057786
Diplosoma listerianum	Aln	2.92767±1.50892	1.08000±0.52289	0.659375±0.425513
Pyura stolonifera	Ind	0.48333±0.42329	57.18208±0.1481	0.415417±0.358929
Actiniaria				
Anthothoe chilensis	Ind	0.57542±0.29146	0.05958±0.04064	0.667917±0.340444
Holothuroidea				
Pentacta doliolum	Ind	0.03167±0.01209	0.03875±0.0137	0.021667±0.019098
Polychaeta				
Autolytus tuberculatus	Ind	0.00083±0.00058	0.00054±0.0004	0.00025±0.00025
Platynereis dumerilii	Ind	0.00996±0.00291	0.04083±0.01278	0.027833±0.014249
Filograna implexa	Ind		0.00083±0.00083	
Spirorbis spp	Unid	0.00092±0.00051	0.00117±0.00088	0.004500±0.003345
Nematoda				
Nematodes	Ind	0.01625±0.01376	0.01383±0.0082	
Cirripedia				
Notomegabalanus algicola	Ind	3.33775±1.33295	2.10242±0.89494	1.706792±0.520136

Bryozoa				
Menipea triseriata	Ind	2.37463±0.80433	2.73750±1.21724	2.283750±0.828028
Bugula neritina	Aln	0.43346±0.21877	0.28275±0.10950	0.566458±0.264652
Hydrozoa				
Obelia dichotoma	Aln	4.98292±1.31183	9.04100±2.48765	4.766625±1.653958
Eudendrium spp	Unid	0.0055±0.00318	0.03867±0.02399	0.013375±0.009238
Rhodophyta				
Ceramium atrorubescens	Ind	0.33838±0.11387	0.52338±0.37268	0.205292±0.084087
Antithamnionella spirographidis	Aln	0.66625±0.49028	0.11625±0.11625	
Lomentaria diffusa	Ind		0.00500±0.00500	
Pachymenia orbitosa	Ind	0.03858±0.01276	0.22208±0.10831	0.12275±0.084328
Delesseria papenfussii	Ind		0.01833±0.01833	0.039583±0.039583
Polysiphonia urbana	Ind	0.05533±0.04569	0.29208±0.20611	0.009417±0.003159
Rhodymenia obtusa	Ind	0.54850±0.249	0.54625±0.41161	0.768958±0.416815
Chlorophyta				
Ulva spp	Unid	0.03642±0.02477	0.11063±0.0554	0.028958±0.016525
Ulva intestinalis	Ind	0.05558±0.04041	0.26792±0.15975	0.09±0.080242
Bioslime	Unid	0.0675±0.04523	0.14788±0.06313	0.140833±0.101392
Papyri looking algae	Unid		0.00383±0.00375	0.010875±0.010832
Rhizoclonium spp	Unid	0.38125±0.19226	0.82463±0.31378	0.533333±0.318945
Caulerpa holmesiana	Ind		0.00208±0.00147	
Ochrophyta				
Desmarestia firma	Ind	0.07958±0.03937	0.01625±0.011	0.040833±0.030467
Nothogenia ovalis	Ind		0.00417±0.00417	0.005833±0.005833
Unidentified				
Orange blobs	Unid	0.00092±0.00045	0.01592±0.01497	0.001333±0.000441

Appendix 3.1

Table 1: Comparisons of co-efficients of the respective factors included in the GLS model for Shannon Wiener and Pielou's evenness diversity indices. ns = non-significant results ($p > 0.05$).

Co-variants	Co-efficient	t-value	p-value
Shannon Wiener diversity index			
Intercept	2.32	51.38	p < 0.0001
Spring	-0.21	-4.10	p < 0.05
Summer	-0.26	-5.24	p < 0.0001
Winter	0.05	0.99	ns
Shallow	-0.02	-0.68	ns
Top	-0.02	-0.62	ns
Pielou's evenness index			
Intercept	0.90	224.53	p < 0.0001
Spring	-0.01	-1.77	ns
Summer	-0.08	-13.54	p < 0.0001
Winter	-0.04	-7.31	p < 0.0001
Shallow	0.02	4.94	p < 0.05

Appendix 3.1 (Continued)

Table 2: Comparisons of co-efficients of the respective factors included in the GLS models for total density (individuals/m^2) and biomass (g/m^2) of all fouling organisms.

Co-variants	Co-efficient	t-value	p-value
Total density			
Intercept	355863.5	8.10	$p < 0.0001$
Spring	217643.8	2.17	$p < 0.05$
Summer	-286882.3	-6.50	$p < 0.0001$
Winter	-139502.1	-2.86	$p < 0.05$
Total biomass			
Intercept	2254.481	8.94	$p < 0.0001$
Spring	749.458	2.64	$p < 0.05$
Summer	19328.198	15.10	$p < 0.0001$
Winter	9275.800	11.99	$p < 0.0001$
Shallow	-808.097	-2.89	$p < 0.05$

Appendix 3.1 (Continued)

Table 3: Comparisons of co-efficients of the respective factors included the GLS model for density (individuals/m^2) and biomass (g/m^2) of alien and indigenous species. ns = not significant ($p > 0.05$).

Taxonomic groups	Co-variants	Co-efficient	t-value	p-value
Indigenous				
	Intercept	1772.31	5.32	p < 0.0001
	Spring	13978.37	4.70	p < 0.0001
	Summer	13600.27	4.70	p < 0.0001
	Winter	895.38	1.85	ns
	Shallow	2270.86	3.64	p < 0.05
	Top	154.74	0.36	ns
	Biomass			
	Intercept	398.54	6.45	p < 0.0001
	Spring	770.95	2.78	p < 0.05
	Summer	212.14	1.76	ns
	Winter	-177.02	-2.81	p < 0.05
	Shallow	289.76	3.76	p < 0.05
	Top	46.16	1.00	ns
Alien				
	Density			
	Intercept	363049.5	8.01	p < 0.0001
	Spring	194984.7	2.04	p < 0.05
	Summer	-307955.9	-6.80	p < 0.0001
	Winter	-148595.4	-2.98	p < 0.05
	Shallow	-7319.4	-1.32	ns
	Biomass			
	Intercept	1606.68	9.64	p < 0.0001
	Spring	245.40	1.58	ns
	Summer	18907.86	14.51	p < 0.0001
	Winter	9295.32	12.85	p < 0.0001
	Shallow	-501.23	-3.12	p < 0.05
	Top	-49.67	-0.33	ns

Appendix 3.1 (Continued)

Table 4: Comparisons of co-efficients of the respective factors included in the GLS model for density (individuals/m^2) and biomass (g/m^2) of the *J. marmorata, S. algosus* and *M. galloprovincialis* samples removed from oyster cages. ns = not significant ($p > 0.05$).

Species	Co-variants	Co-efficient	t-value	p-value	Co-variants	Co-efficient	t-value	p-value
Ciona intestinalis								
	Density				*Biomass*			
	Intercept	730.57	3.34	p < 0.05	Intercept	1269.35	2.56	p < 0.05
	Shallow	-95.98	-0.48	ns	Shallow	194.65	0.55	ns
	Top	-231.92	-1.06	ns	Top	-675.84	-1.33	ns
Jassa marmorata								
	Density				*Biomass*			
	Intercept	338594.0	5.35	p < 0.0001	Intercept	377.5492	5.01	p < 0.0001
	Spring	-292500.6	-4.55	p < 0.0001	Spring	-316.1117	-4.11	p < 0.05
	Summer	-338286.2	-5.35	p < 0.0001	Summer	-377.3384	-5.01	p < 0.0001
	Winter	-333732.9	-5.27	p < 0.0001	Winter	-368.1516	-4.89	p < 0.0001
	Shallow	2852.9	2.02	p < 0.05	Shallow	4.8980	2.25	ns
	Top	575.6	0.88	ns	Top	0.2945	0.67	ns
Semmytilus algosus								
	Density				*Biomass*			
	Intercept	3820.1	8.15	p < 0.0001	Intercept	476.1001	6.50	p < 0.0001
	Spring	379442.7	6.13	p < 0.0001	Spring	833.8896	6.00	p < 0.0001
	Summer	697.5	2.23	p < 0.05	Summer	1403.2111	6.50	p < 0.0001
	Winter	114358.8	6.65	p < 0.0001	Winter	2687.8316	6.85	p < 0.0001
	Shallow	-1926.2	-4.54	p < 0.0001	Shallow	-292.6339	-4.15	p < 0.05
	Top	-956.4	-2.86	p < 0.05	Top	-90.2170	-2.08	p < 0.05
Mytilus galloprovincialis								
	Density				*Biomass*			
	Intercept	10395.51	8.81	p < 0.0001	Intercept	667.075	5.26	p < 0.0001
	Spring	120220.12	5.31	p < 0.0001	Spring	-185.435	-1.45	ns
	Summer	38991.70	6.50	p < 0.0001	Summer	17948.835	15.02	p < 0.0001
	Winter	77452.54	5.84	p < 0.0001	Winter	2846.023	7.56	p < 0.0001
	Shallow	-3825.91	-3.42	p < 0.05	Shallow	-169.273	-1.35	ns
	Top	-2054.69	-2.14	p < 0.05				

Appendix 3.2: A list of biomass (g/plate ± 1 SE) of fouling species present on oyster cages sampled during the four seasons. Aln= Alien species, Ind= Indigenous species, Unid= Unidentified species and Cryp= Cryptogenic species.

Taxonomy		Status	Summer	Autumn	Winter	Spring
Amphipoda	*Caprella equlibria*	Cryp	0.10±0.043	9.3042±5.03714	25.260±14.2974	0.892±0.2450
	Paramoera capensis	Ind	18.94±7.407	3.0427±0.74167	17.594±4.7889	57.656±10.8994
	Jassa marmorata	Aln	2.33±0.872	383.0938±65.83040	13.823±5.7307	62.750±9.4420
	Jassa slatteryi	Aln	0.00±0.00	4.6979±1.20911	0.00±0.00	0.00±0.00
	Lysianassa ceratina	Ind	0.10±0.104	0.00±0.00	0.042±0.0417	0.00±0.00
Tanaidacea (Tanaids)	*Anatanais gracilis*	Ind	16.09±2.120	1.7281±0.29920	1.497±0.2908	1.552±0.2422
Isopoda	*Sphaeramene polytylotos*	Ind	1.38±0.584	0.6146±0.38069	0.063±0.0458	0.00±0.00
	Exosphaeroma laeviusculum	Ind	0.00±0.00	0.0042±0.00417	0.00±0.00	0.00±0.00
	Paridotea reticulata	Ind	0.00±0.00	1.6979±0.63950	2.427±1.5466	4.177±1.1044
Decapoda	*Hymenosoma orbiculare*	Ind	0.01±0.004	1.4813±1.02303	2.146±0.6325	1.740±0.6644
	Jasus lalandii	Ind	0.00±0.00	0.00±0.00	0.00±0.00	0.406±0.4063
Bivalvia	*Venerupis corrugatus*	Ind	25.44±5.616	9.0302±2.46129	5.302±1.3341	0.167±0.0860
	Mytilus galloprovincialis	Aln	18545.91±1091.787	604.2292±84.43191	3430.403±421.7925	374.573±86.2515
	Crassostrea gigas	Aln	0.00±0.00	0.00±0.00	0.229±0.0519	0.00±0.00
	Aulacomya ater	Ind	0.50±0.162	0.2167±0.07696	1.553±0.5154	0.031±0.0313
	Semimytilus algosus	Aln	1725.86±179.483	254.8438±35.48894	2939.524±399.9250	1208.541±177.6828
Opisthobranchia (Nudibranch)	*Janolus capensis*	Ind	0.00±0.00	0.00±0.00	0.00±0.00	0.073±0.0729
Ascidiacea	*Microcosmos*	Aln	0.20±0.198	0.5146±0.26697	0.010±0.0104	0.00±0.00
	Botryllus magnicoecus	Ind	0.00±0.00	0.4896±0.48958	0.00±0.00	0.00±0.00
	Diplosoma listerianum	Aln	0.00±0.00	0.00±0.00	0.00±0.00	3.729±2.1604
	Ciona intestinalis	Aln	17.60±15.901	18.5625±9.19184	4188.385±805.3024	4.302±2.1553
	Pyura capensis	Ind	10.79±6.854	1.4688±0.71969	0.00±0.00	2.344±0.9951
Actiniaria (Anemone)	*Pseudactinia flagellifera*	Ind	0.01±0.010	1.1979±1.19792	1.104±0.8721	0.00±0.00
	Bunodosoma capensis	Ind	0.00±0.00	0.3229±0.32292	3.354±0.5972	0.00±0.00
Holothuroidea (Sea cucumbers)	*Pentacta doliolum*	Ind	12.60±3.071	0.00±0.00	0.003±0.0031	0.003±0.0031
Polychaeta	*Ceratonereis erythraceensis*	Ind	0.41±0.406	0.00±0.00	0.340±0.2013	0.00±0.00
	Nereis spp	Ind	0.19±0.105	0.0208±0.02083	6.938±3.0289	0.00±0.00
	Eulalia	Ind	0.04±0.023	0.00±0.00	0.00±0.00	0.00±0.00
	Syllidae spp	Ind	0.03±0.016	0.0010±0.00104	0.00±0.00	0.00±0.00
	Thelepus spp	Ind	0.19±0.177	0.0052±0.00521	0.004±0.0042	0.010±0.0065
	Platynereis dumerilii	Ind	0.55±0.175	2.7708±0.57023	4.626±0.8307	0.844±0.3401
	Leidonotus semitectus	Ind	19.15±3.739	0.2188±0.14458	1.531±0.3893	0.011±0.0104
Nematoda	*Nematodes*	Ind	1.13±0.344	3.1771±0.55219	16.427±2.9306	0.250±0.1354
Pycnogonida (Sea spiders)	*Tanystylum brevipes*	Ind	0.002±0.002	0.00±0.00	0.042±0.0246	0.001±0.001
Cirripedia (Barnacles)	*Notomegabalanus algicola*	Ind	833.83±150.565	432.9167±41.25293	358.740±45.4892	1025.438±271.3836
Caridea (Shrimp)	*Palaemon peringueyi*	Ind	0.00±0.00	0.2708±0.27083	0.00±0.00	0.00±0.00
Bryozoa	*Bugula neritina*	Aln	1.38±1.375	0.0313±0.03125	0.615±0.6146	0.948±0.8179
	Menipea triseriata	Ind	0.00±0.00	0.00±0.00	0.00±0.00	10.375±3.2385
Echinoidea (Sea urchin)	*Parechinus angulosus*	Ind	0.00±0.00	0.00±0.00	0.016±0.0075	0.00±0.00
Rhodophyta	*Polysiphonia urbana*	Ind	0.00±0.00	0.0313±0.03125	0.00±0.00	0.00±0.00
	Ceramium atrorubescens	Ind	0.00±0.00	0.00±0.00	0.00±0.00	7.396±5.4601